SCIENTIFIC PAPERS

SERIES B. HORTICULTURE

VOLUME LXI, 2017

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

SCIENTIFIC PAPERS
SERIES B. HORTICULTURE

Volume LXI

2017
BUCHAREST

PUBLISHERS:

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

Address: 59 Marasti, District 1, 011464 Bucharest, Romania

E-mail: journal@horticultura-bucuresti.ro, Webpage: www.horticultura-bucuresti.ro

CERES Publishing House

Address: 1 Piaţa Presei Libere, District l, Zip code 013701, Bucharest, Romania

Phone: + 40 21 317 90 23, E-mail: edituraceres@yahoo.com, Webpage: www.editura-ceres.ro

To be cited: Scientific Papers. Series B. Horticulture, Vol. LXI, 2017

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

International Database Indexing: CABI, Index Copernicus (5,17), CNCSIS B+, Ulrich's Periodicals Directory, Google Scholar, PBN (Polish Scholarly Bibliography), Scipio, Cite Factor (Academic Scientific Journals), Scientific Indexing Service, OCLC (WorldCat), Reasearch Bible, Universal Impact Factor

SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARHITECTURE

MISCELLANEOUS

FRUIT
GROWING

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOME POMOLOGICAL AND CHEMICAL PROPERTIES OF LOCAL PEAR VARIETIES IN UŞAK, TURKEY

Volkan OKATAN[1], Mehmet POLAT[2], Sezai ERCİŞLİ[3], Mehmet Atilla AŞKIN[2]

[1]Usak University, Sivasli Vocational School, Uşak, Turkey
[2]Suleyman Demirel University, Agricultural Faculty, Department of Horticulture, Isparta, Turkey
[3]Ataturk University, Agricultural Faculty, Department of Horticulture, Erzurum, Turkey

Corresponding author email: okatan.volkan@gmail.com

Abstract

This study described some desirable pomological and chemical traits of 10 local pear varieties from Uşak in 2016. In these local pear varieties, some pomological and chemical properties were determined such as fruit weight, fruit height, fruit widths, fruits firmness, total soluble solids, titratable acidity, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free-radical values and ascorbic acid content. The values for fruit weights ranged from 93.12 ± 3.26 g to 287.93 ± 8.36 g, fruit height ranged from 6.12 ± 1.37 cm to 11.62 ± 1.15 cm, fruit widths ranged from 4.38 ± 0.82 cm to 8.52 ± 0.63 cm, fruits firmness ranged from 4.26 ± 0.18 lb to 11.19 ± 0.32 lb total soluble solid contents ranged from % 8.62 ± 0.32 to % 17.20 ± 0.83 and, titrable acid contents ranged from % 0.43 to % 2.63. DPPH values were determined and varied from 12.37 ± 0.73 to 26.74 ± 1.68; ascorbic acid content situated between 108.53 ± 3.12 and 263.37 ± 4.71 mg. We conclude that some local pear varieties may be recommended for the next breeding studies.

Key words: *local, pear, dpph, pomological, chemical.*

INTRODUCTION

Due to the favorable climate and soil conditions that our country has, it has the chance to grow a large number of species and varieties in terms of fruit.

Today, Turkey is one of the important fruit producer countries of the world in terms of fruit variety and number of varieties and production amount (Güleryüz, 1977; Özbek, 1977).

The number of pear varieties in the world is over 5000 and this number is 600 in Turkey. However, among them, the number of those are high in quality and productivity, and cultivated commercially (Buyukyilmaz, 1993).

Pear is the most mild climate fruit species after apple in the world. Pear fruit can be consumed both fresh and dry. It is also used in pear liquor, vinegar, fruit juice, fruit salad, jam, jelly, dessert, cake, pastry and canned food industry (Özçağıran et al., 2005).

The aim of this research was to determine the some pomological and chemical properties of local pear varieties grown in Uşak region in Middle Eagean of Turkey.

MATERIALS AND METHODS

The climate of the Uşak province shows a transition characteristic between the Aegean and Central Anatolian regions. More continental climate prevails. The summers are warm, the winters are long and hard.

The annual rains is between 430 mm and 700 mm. Most of the rains fall in winter. Summer rainfall is rather low.

The research district is situated between 38°40′ North latitude and , 29°23′ East longitude in Middle Eagean of Turkey. Uşak region is nearly 906 meters above sea level (Wikipedia participants, 2017).

The data of microclimates of the research district are presented Table 1.

Fruit material

Local pear genotypes were selected according to fruit sizes. Ten local pear cultivars were harvested in different region of Uşak, Middle Eagean, Turkey, in Semptember-October 2016.

The harvested fruits were then transported to the laboratory for analysis.

Table 1. Average monthly air temperature (°C) and total rainfall (kg/m^2) in the Uşak – 1916 to 2016.

USAK	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Maximum (°C)	6.8	8.1	12.0	17.0	22.0	27.0	30.0	31.0	26.0	20.0	14.0	8.7
Minimum (°C)	-1.3	-1.0	1.3	5.2	9.2.0	13.0	15.0	16.0	12.0	7.9	3.8	0.6
Rainfall (kg/m^2)	75.0	66.0	59.0	50.0	48.0	28.0	15.0	9.6	16.0	40.0	59.0	82.0

(Source: www.mgm.gov.tr)

Methods

The matured fruits were selected based on morphological characteristics for fruit analyses. Desirable morphological characteristics such as fruit weight (g), fruit height (mm), fruit weight (mm), fruit firmness (lb), total soluble solid content (SSC,%) and acidity (%) were determined according to Cemeroglu (1992).

Determination of vitamin C

After pureeing and filtering, the fruit juices samples were obtained. The juices were used for vitamin C analysis.

The samples were homogenized by centrifuge and 400 µL oxalic acid (0.4 %) and 4.5 ml 2,6 - diclorofenolindofenol solution were added upon supernatant.

The data were read spectrophotometrically at the wavelength of 520 nm against the blank.

Determination of radical – scavenging activity:

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

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

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

RESULTS AND DISCUSSUION

The fruit weight (g), fruit height (mm), fruit width (mm) and fruit firmness (lb) measurement in fruits of 10 local pear genotypes were examined in this study (Table 2).

According to the measurements, among pear genotypes were found to be; for fruit weights ranged from 93,12 ± 3,26 g to 287,93 ± 8,36 g, fruit heights ranged from 6,12 ± 1,37 cm to 11,62 ± 1,15 cm, fruit widths ranged from 4,38 ± 0,82 cm to 8,52 ± 0,63 cm, fruits firmness ranged from 4,26 ± 0.18 lb to 11,19 ± 0,32 lb average.

In the study on pear varieties in Yalova, there were identified fruits in diameters ranging from 42.61 to 83.54, fruit heights ranging from 72.83 to 108.25, fruit weight ranging from 57.26 to 410.75 g, SSC between 11.17 and 14.06 and TA 0.23 to 0.40 g / 100 ml (Akçay et al., 2009). In pear fruit which is very rich in nutritional value, the amount of SSC is 14.63-19.5 % and the titratable acidity value is 0.154-0.462 % (Özbek, 1978; Şen et al., 1992).

Table 2. Pomological characteristics of pear genotypes

Genotypes	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Fruit firmness (lb)
64 USAK 01	117.26 ± 5.13	9.52 ± 0.83	5.24 ± 0.68	5.19 ± 0.14
64 USAK 02	287.93 ± 8.36	11.62 ± 1.15	8.52 ± 0.63	7.26 ± 0.56
64 USAK 03	185.83 ± 6.19	6.12 ± 1.37	6.81 ± 0.92	6.84 ± 0.27
64 USAK 04	114.85 ± 9.12	7.26 ± 1.08	4.97 ± 0.47	4.26 ± 0.18
64 USAK 05	247.51 ± 12.75	8.46 ± 0.72	7.16 ± 0.54	9.10 ± 0.59
64 USAK 06	93.12 ± 3.26	10.36 ± 0.78	4.38 ± 0.82	8.74 ± 0.47
64 USAK 07	176.28 ± 15.16	11.35 ± 1.03	8.26 ± 1.04	6.57 ± 0.26
64 USAK 08	139.68 ± 7.28	6.28 ± 0.47	5.26 ± 0.41	11.19 ± 0.32
64 USAK 09	162.44 ± 6.17	8.51 ± 1.25	6.29 ± 0.49	10. 23 ± 0.55
64 USAK 10	192.74 ± 9.84	9.84 ± 0.42	8.06 ± 0.82	9.61 ± 0.92

SSC, TA, DPPH and ascorbic asid measuretments in genotypes are presented in Table 3. According to the measurements, among pear genotypes were found to be; for total soluble solid contents ranged from % 8.62 ± 0.32 to % 17.20 ± 0.83 and, titrable acid contents ranged from % 0.43 ± 0.05 to % 2.63 ± 0.12. DPPH values were determined between from 12.37 ± 0.73 to 26.74 ± 1.68 and ascorbic acid content were found between 108.24 ± 3.12 and 263.36 ± 4.71 mg average.

Table 3. Chemical characteristics of pear genotypes.

Genotypes	SSC %	TA %	DPPH	Ascorbic Acid (mg)
64 USAK 01	12.84 ± 0.23	2.63 ± 0.06	21.82 ± 1.26	147.18 ± 2.15
64 USAK 02	16.15 ± 0.56	1.79 ± 0.05	12.37 ± 0.73	162.93 ± 5.24
64 USAK 03	9.62 ± 0.08	1.52 ± 0.09	17.19 ± 1.38	108.53 ± 3.12
64 USAK 04	8.62 ± 0.32	0.82 ± 0.01	16.25 ± 1.24	125.28 ± 4.81
64 USAK 05	11.69 ± 1.24	0.91 ± 0.02	18.22 ± 0.86	263.37 ± 4.71
64 USAK 06	15.27 ± 0.85	0.43 ± 0.15	24.15 ± 1.76	214.26 ± 7.59
64 USAK 07	13.60 ± 0.16	1.35 ± 0.09	26.74 ± 1.68	185.21 ± 4.19
64 USAK 08	17.20 ± 0.83	2.14 ± 0.27	18.21 ± 1.25	192.17 ± 9.14
64 USAK 09	16.21 ± 1.54	2.37 ± 0.11	17.91 ± 1.17	251.66 ± 4.10
64 USAK 10	15.28 ± 0.84	2.18 ± 0.29	13.57 ± 1.19	182.24 ± 6.43

According to the average of the two years in the examined varieties; the total soluble solids ranged from 10.6% (Etrusc) to 15.75% (Hosui). In studies similar to this, the total soluble solid ratio was 14.60% - 19.90% (Güleryüz, 1972). In a study on pear varieties in JAPAN, They were identified as high DPPH radical scavenging activity 19.76% (Ieguchi et al., 2015). Szete et al. (2002) reported an average for ascorbic acid of 60 mg in a study of pear varieties in China.

CONCLUSIONS

As a result, fruit growing culture is based on very ancient histories in Uşak province. For centuries, many civilizations and a large number of local fruit genotypes have hosted in this region, so there has an important selection potential.

REFERENCES

Akçay, M. E., Büyükyılmaz, M., & Burak, M. (2009). Marmara Bölgesi için ümitvar armut çeşitleri-IV. Bahçe, 38(1), 1-10.

Blois M. S., 1958. Antioxidant determinations by the use of a stable free radical, Nature, 181, 1199 - 1200.

Büyükyılmaz, M. Armut çeşit kataloğu. T.C. Tarım ve Köyişleri Bakanlığı Yayın No: 360/19. Ankara 47 s. 1993.

Cemeroglu B: Meyve ve Sebze İşleme Endüstrisinde Temel Analiz Metodları. Ankara: Biltav Yayınları; 1992:381.

Güleryüz, M., 1972. Erzincan'da Yetiştirilen Bazı Önemli Elma ve Armut Çeşitlerinin Pomolojileri ile Döllenme Biyolojileri Üzerinde Araştırmalar, Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, Erzurum, 216 s.

Güleryüz, M., 1977. Erzincan'da Yetiştirilen Bazı Önemli Elma ve Armut Çeşitlerinin Pomolojileri ve Döllenme Biyolojileri. Atatürk Üniversitesi Ziraat Fakültesi, Yayın No:229, Erzurum.

Gulcin I., 2007. Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa, Amino acids, 32: 431-438.

Ieguchi, T., Takaoka, M., Nomura, K., Uematsu, C., & Katayama, H. (2015). Pear (Pyrus L.) Genetic Resources from Northern Japan: Evaluation of Antioxidant capacity. Acta Horticulturae.

Özbek, S., 1977. Genel Meyvecilik. Çukurova Üniversitesi Yayınları No: 111, Adana.

Özbek, S., 1978. Özel Meyvecilik. Çukurova Üniv. Ziraat Fak. Yayın No:128, Adana. 486 s

Özçağıran, R., Ünal, A., Özeker, E., ve İsfendiyaroğlu, M., 2005. Ilıman İklim Meyve Türleri. Cilt-3, Ege Üniversitesi Yayınları, İzmir.

Szeto, Y. T., Tomlinson, B., & Benzie, I. F. (2002). Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. British journal of nutrition, 87(01), 55-59.

Şen, S. M., Cangi, R., Bostan, S. Z., Balta, F., Karadeniz, T., 1992. Van ve çevresinde yetiştirilen seçilmiş bazı Mellaki ve Ankara armut çeşitlerinin fenolojik, morfolojik ve pomolojik özellikleri üzerinde araştırmalar. Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Dergisi, 2(2):29-40.

Wikipedia participants (2017). Servant. Wikipedia, The Free Encyclopedia. Access date 20:00, April 4, 2017 url: //en.wikipedia.org/wiki/U%C5%9Fak

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECTS OF RELATIVE HUMIDITY ON *IN VITRO* POLLEN GERMINATION AND TUBE GROWTH IN SWEET CHERRIES (*PRUNUS AVIUM* L.)

Sultan Filiz GÜÇLÜ[1], Fatma KOYUNCU[2]

[1]Süleyman Demirel University, Atabey Vocational School, Isparta, Turkey
[2] Süleyman Demirel University, Faculty of Agriculture, Department of Horticulture, Isparta, Turkey

Corresponding author email: sultanguclu@sdu.edu.tr

Abstract

Objective of this study was to examine the effects of global warming on pollen germination in fruit trees. For this purpose Bigarreu Gaucher, Celeste, Lambert, Lapins, Starks Gold, Stella and 0900 Ziraat cherry cultivar's pollens were used. Pollen germination and tube growth rates were observed at 40%, 50%, 60%, 70%, 80% and 90% relative humidity regimes. High humidity regimes were gave best results for both pollen germination and pollen tube growth. 80% was the optimum RH for in vitro germination of Starks Gold and Stella while 90% was optimum for Bigarreu Gaucher. In vitro pollen germination increased with increasing incubation relative and incubation period. 24 hours later was the optimum for all cultivars. The longest pollen tube length was obtained from 90% RH. Set fruitful could be affected by relative humidity and indirectly global warming.

Key words: *relative humidity, pollen germination, Prunus avium* L.

INTRODUCTION

Environmental conditions play a key role for the survival living organisms (Koubourıs et al., 2009). The environment can be an important determinant of pollen performance.
The effects of the environment on pollen germination may be broken down into three categories: (1) prepollination environment, (2) pollination environment, and (3) postpollination environment. The prepollination environment consists of the environmental conditions in which a pollen-donating plant grows. Pollen may also be influenced by the environment experienced during pollen transfer from donor to recipient (Travers, 1999).
Fertilization success in plants is the result of processes that take place during the progamic phase. Recently studies show that environmental conditions affect pollen devolopment and pollen germination as well as pollen tube growth.
Enviromental conditions affect steps such as pollen germination and pollen tube growth as well as development of the female structures (Stephenson et al., 1992; Delph et al., 1997; Hedly et al., 2004).
Among those environmental conditions, temperature is one of the most important factors that affect fruit and seed set. Another factor of pollen germination and tube growth is relative humidity.
Humidity and temperature has been shown to affect the chemical composition of pollen, pollen viability, and/or pollen tube growth and to stimulate the synthesis of heat and shock proteins in pollen grains (Johannson and Stephenson, 1998).
Pollen germination and pollen tube growth are important components of fertilization success in fruit trees (Janıck and Moore, 1996; Tosun (Güçlü) and Koyuncu, 2007).
Few studies, however, have examined both the environmental and the genetic effects of temperature and humidity on pollen performance (Loupassaki et al., 1997).
Temperature and humidity has a clear effect on pollen tube kinetics, expressed as the time required for pollen germination and rate of tube growth. The pollen germination and pollen tube growth are the most important properties in cherry tree fertilization. Living organisms depend on water. Water regulates their biological reactions, serves as a fluid medium and stabilizes the structure of macromolecules.

Although heavy water loss from living organisms may have deleterious effects and may lead to death (Alper and Olıver, 2002; Nepı et al., 2010).
Recent years global warming effects direct pollen germination, pollination timing, and pollen tube growth and set fruitful. We think that it is effected indirectly, reducing relative humidity. Relative humidity is very important for pollen germination stage. Partial dehydration brigs the pollen into equilibrium with the environment without fatal damage to the cytoplasm (Bassani et al., 1994) however uncontrolled water loss induced by high temperature leads to the death of the pollen (Pacini, 1996; Koubouris et al., 2009). We think that humidity rate can be affected by high temperature and indirectly fruit set can be changed.
In this study we try to explain effects of humidity on pollen germination and tube growth in sweet cherries which are grown extensively in Turkey.

MATERIALS AND METHODS

'Bigarreu Gaucher', 'Celeste', 'Lambert', 'Lapins' 'Starks Gold', 'Stella', and '0900 Ziraat' cherry cultivars were used for germination tests. Flowers at balloon stage were collected from plants in early morning. The flowers were transferred to the laboratory quickly. The anthers were removed and placed in dark colored bottles to promote dehiscence at room temperature. The 'agar in plate' method was used to assign pollen germination and pollen tube growth.
Pollen grains were sown in the medium containing 0.5 agar + 15% sucrose + 5 parts per million H_3BO_3 (Boric acid) were put consist of 40%, 50%, 60%, 70%, 80%, 90% humidity cabins at 25°C. Pollen tube long at least as its diameter was considered to be 'germinated' (Tosun (Güçlü) and Koyuncu, 2007).
The percentage of germinating pollen was determined after 2h, 6h, 12h and 24h incubation period. An ocular micrometer was used to measure pollen tube lenght, under a light microscope, at a magnification 40X. Four Petri dishes were used for germination and pollen tube growth experiments. Counts were made from 4 different microscope fields (100-150 pollen grains per field) in each Petri dish. In all experiments, treatments were arranged according to randomized design.
Statistical analyses were performed with GLM models (General Linear Model) using SPSS (V.10; Statistical software, SPPS. Inc., USA). Percentage data were subject to arcsin root square transformation, and analysis of variance was performed. The differences among means were analysed by Duncan's multiple range test at the 0.05 level of significance.

RESULTS AND DISCUSSIONS

Pollen germination rates of seven sweet cherry cultivars are shown in Table 1 after 24 hours at different humidity regimes (40% RH, 50% RH, 60% RH, 70% RH, 80% RH, 90% RH).

Table 1. *In vitro* germination (%) of cherries pollen at different humidity regimes after 24 hours incubation, in a medium containing 0.5 agar + 15% sucrose + 5parts per million H_3BO_3

	Humidity regimes					
Cultivar	40%	50%	60%	70%	80%	90%
Bigarreu Gaucher	4.12a	11.23a	35.30a	54.09b	82.30b	91.69a
Celeste	1.33b	3.19c	14.29d	51.30c	72.46d	81.88b
Lambert	1.09b	2.00d	25.12c	46.76d	71.87d	83.45b
Lapins	1.25b	3.76c	12.30d	54.63b	76.56c	79.83b
Starks Gold	1.79b	6.90b	29.96b	55.88b	88.46a	70.05c
Stella	1.88b	3.01c	23.62c	64.03a	89.76a	71.92c
0900 Ziraat	1.03b	3.00c	8.87e	45.56d	74.36c	66.25d

Different letters in the same column indicate significant differences, according to Duncan's multiple range test (P<0.05).

All cultivars showed highest germination in 90% RH except for 'Bigarreu Gaucher'.'Noble' and '0900 Ziraat'. The lowest pollen germination rates were obtained from 40% RH. There is no difference statistically ($p<0.05$) except from 'Bigarreu Gaucher'.
Pollen germination rates increase with rising relative humidity. 'Bigarreu Gaucher' had the best germination rate both 50% and 60% RH. '0900 Ziraat' have had the lowest value with 8.87 germination rate at 60% RH ($p<0.05$).
The best results obtained from 80% and 90% RH. 80% was the optimum RH for 'Starks

Gold' and 'Stella' while 90% was optimum for 'Bigarreu Gaucher'.

It can be said that '0900 Ziraat' showed the lowest germination rates in general. Germination of cherry pollens reached own maximum percentage in 24 hours for all relative humidity regimes (Figure1).

Pollens of '0900 Ziraat' and 'Lapins' started latest germinated at 40% RH. Pollens of these cultivars germinated 24 hours later. 'Starks Gold' pollens germinated 2 hours later at 40% RH different from other cultivars (0.23). After 12 hours pollen germination rate in Celeste showed a dramatically increase from 13.96 to 69.98.

The pollen tube elongation is showed in Table 2 according to different humidity regimes.

Table 2. The effect of relative humidity on pollen tube growth (µm) cherry cultivars *in vitro* after 24 hours

	Humidity regimes					
Cultivar	40%	50%	60%	70%	80%	90%
Bigarreu Gaucher	17.96a	35.58a	52.29a	82.35a	116.33a	119.22a
Celeste	6.98c	16.23c	39.96c	69.73b	80.11c	89.74d
Lambert	5.11c	13.29d	26.34de	58.96c	71.23d	92.33d
Lapins	6.74c	15.71c	30.33d	63.72bc	88.9c	100.01c
Starks Gold	10.12b	21.79b	43.25b	66.12b	93.7b	110.23b
Stella	6.03c	14.1c	29.91d	56.73c	71.32d	90.83d
0900 Ziraat	4.79cd	10.11e	21.03e	30.54d	62.23e	80.09e

Different letters in the same column indicate significant differences, according to Duncan's multiple range test ($p<0.05$).

Results were found statistically significant ($p<0.05$). Pollen tube growth increased with increased humidity. All cultivars showed longest pollen tube growth at 90% relative humidity and 24 hours incubation period.

The longest pollen tubes were obtained from 'Bigarreu Gaucher' at all temperatures while the shortest were found for '0900 Ziraat'. 'Lambert' showed the lowest pollen tube elongation from 40% RH to 90% RH.

All cultivars showed different response for relative humidity. Oyiga et al. (2010), reported that pollen germination is species depend on environmental factors. Another study which was carried out in olive cultivars showed extreme temperature and relative humidity incidents, even for a short period, reduced pollen germination and growth capacity in 'Koroneiki', 'Kalamata', 'Mastoidis' and 'Amigdalolia' olive and may affect fruit set and yield (Koubourıs et al., 2009). *Petunia hybrida* and *Cucurbita pepo* pollens were exposed to 30 and 75% relative humidity (RH).

Water content, viability and carbohydrate content (glucose, fructose, sucrose and starch) were measured at fixed intervals over 6 h. Water content of *C. pepo* pollen decreased drastically at both RH, while *P. hybrida* pollen dehydrated slightly at RH 30% and hydrated at RH 75% (NEPI et al., 2010). 24 hours later pollen germination reached maximum.

These results of incubation duration experiments were closely parallel to findings of Koyuncu (2006), who reported that germination of strawberry pollens began within 1h at 24°C. Similarly, the pollen of 'Tsanoki' pear started to germinate after 1h incubation (Vasilikakis and Porlingis 1985). Koyuncu and Tosun (Güçlü) (2008) reported that the germinating rates increased with length of incubation period. Leech et al. (2002), investigated the responses of a period of up to 72 hours on pollen germination of different strawberry cultivars. The growth of the pollen tube in flowering plants is exceedingly rapid and its requirements, in general, seem quite unimpressive, i.e., water, oxygen and a suitable osmotic environment. Despite extensive attempts to hasten this growth process with the conventional host of growth factors, few have met with convincing success (Brewbaker and Kwack, 1963).

In vivo pollen germination and tube growth are highly sensitive to climatic factors, particularly, temperature and relative humidity (Sıngh at al., 2009). While temperature and other abiotic stresses are clearly limiting factors for crops cultivated on marginal lands, crop productivity everywhere is often at the mercy of random environmental fluctuations. Current speculation about global climate change is that most agricultural regions will experience more extreme environmental fluctuations (Solomon et al., 2007; Zinn et al., 2010). On our opinion climatic change can affect relative humidity, pollen germination, bee activity and fruitset.

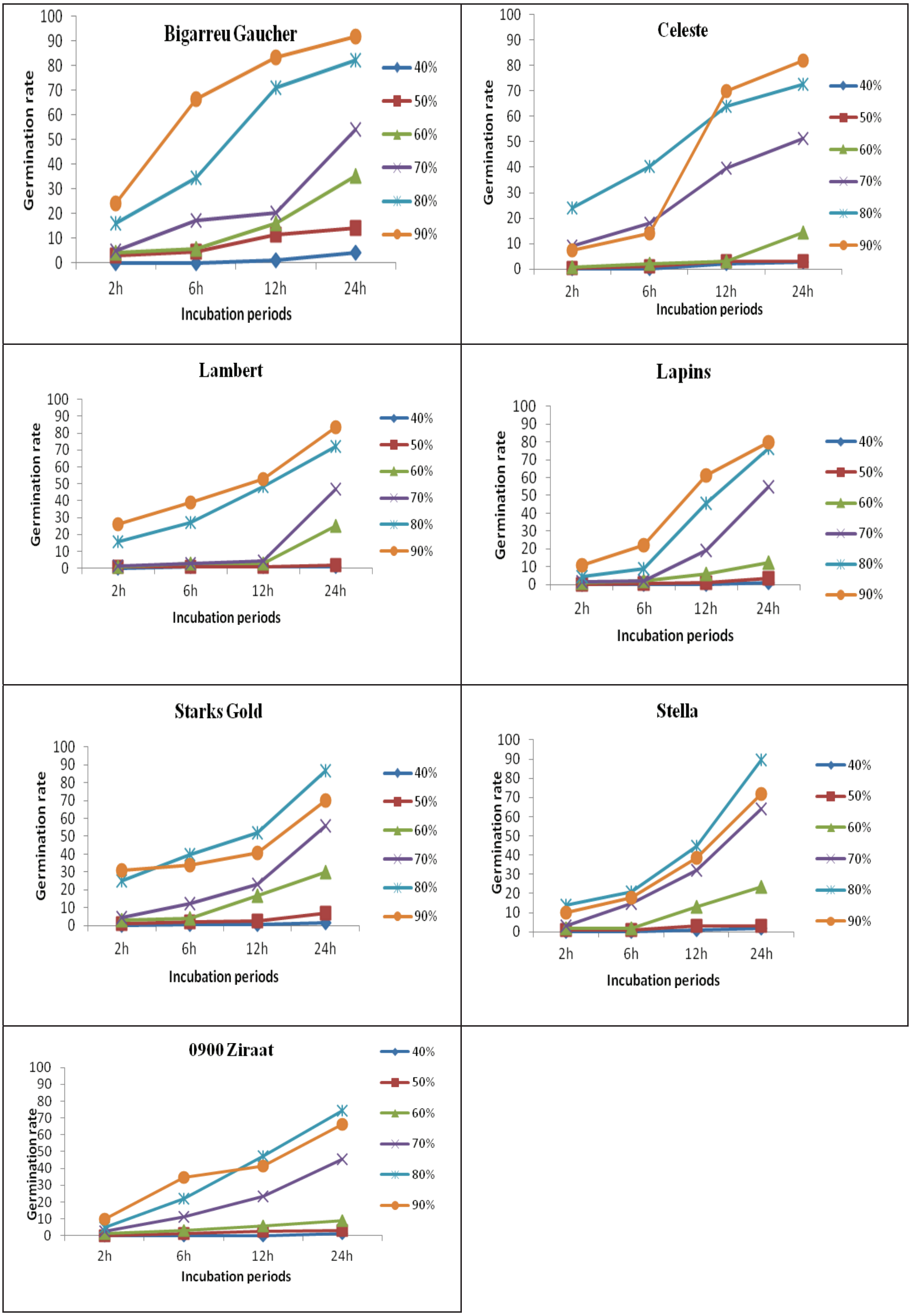


Figure 1. Germination of different cherry varieties pollens

CONCLUSIONS

Indirect effects of global warming on pollen germination and fruit set has examined in this study.

Pollen germination and pollen tube growth reached own maximum percentage at high humidity regimes.

'Starks Gold' and 'Stella' gave best resutls at 80% humidity.

90% humidity was optimum for 'Bigearreu Gaucher'.

Highest percentage was taken at 24 hours later after incubation for all cultivars.

Pollen germination and tube growth increased by incubation period.

On our opinion climatic change can affect relative humidity, pollen germination, bee activity and fruitset.

REFERENCES

Alper P., Oliver M.L(2002). Drying without dying. In: Black M, Pritchard HW (eds) Desiccation tolerance in plants. CAB International, Wallingford, pp 3–43.Assessment Report of the Intergovernmental Panel on Climate Change. New York: Cambridge University Press.

Bassanı M.,. Pacın E., Franchı G.G., 1994. Humidity stress responses in pollen of anemophilous species. Grana, 33, 146-150.

Brewbaker, J.L. and Kwack H.B 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Am J Bot, 50 (9), 747-858.

Delph L., Johannson F. and Stephenson A.G., 1997. How environmental factors affect pollen performance, ecological and evolutionary perspectives. Ecology. 78, 1632-1639.

Hedly A.. Hormaza J.I and Herrero M., 2004. Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (*Rosaceae*). Am J Bot 91 (4), 558-564.

Janıck J., Moore N.J., 1996. Fruit Breeding, Tree and Tropical Fruits Vol. 1. New York, John Wiley and Sons Inc

Johannson L.F. and Stephenson A.G., 1997. How enviromental factors affect pollen performance; ecological and evolutionary perspectives. Ecology 78, 1632-1639.

Johannson H.M. and Stephenson G.A. 1998. Effect of temperature during microsporogenesis on pollen performance in *Cucurbita pepo* L. (*Cucurbitacea*). Int J Plant Scı, 159 (4), 616-626.

Koubourıs C.G., Metzıdakıs T.I. and Vasılakakıs D.M., 2009. Impact of temperature on olive (*Olea europaea* L.) pollen performance in relation to relative humidity and genotype. Envıron Exp Bot 67, 209–214.

Koyuncu F. and. Tosun (Guclu) F 2008. Evaluation of pollen viability and germinating capacity of some sweet cherry cultivars grown in Isparta, Turkey. Acta Hort.795 ISHS, (1), 71-75.

Koyuncu F., 2006. Response of *in vitro* pollen germination and pollen tube growth of strawberry cultivars to temperature. Europ J. Hor. Sci. 71(3), 125-128.

Leech L.D,, Simpson W. and Whıtehouse A.B., 2002. Effect of temperature and relative humidity on pollen germination in four strawberry cultivars. Acta Hort. 567 ISHS, 261-263

Loupassakı M., Vasılakakıs M., and Androulakıs, I. 1997. Effect of Pre-incubation Humidity and Temperature Treatment on the Germination of Avocado Pollen Grains. Euphytica, 94, 247-251.

Nepı M., Cresti. N.. Guarnıerı M. and Pacını E., 2010. Effect of relative humidity on water content, viability and carbohydrate profile of *Petunia hybrida* and *Cucurbita pepo* pollen. Plant System Evolution, 284, 57–64.

Oyiga B.C., Uguru M.I. and Aruah C.B., 2010. Pollen behaviour and fertililization impairment in Bambara groundnut (*Vigna subterrenea* [L.] Verdc.). JPBCS 2(1), 12-23.

Pacını E., 1996. Types and meaning of pollen carbonhydrate reserves. Sex Plant Reprod, 9, 362-366.

Sıngh S., Rana A. and Chauan S.V.S. 2009. Impact of environmental changes on the reproductive biology in *Pyrostegia venusta* Presl. J Environ Biol, 30 (2), 271-273.

Solomon S., Qın D, Mannıng M., Marquıs M., Averyt K., Tignor M.M.B., Mıller H.L and Cheng Z. 2007. Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth

Stephenson A., Lau T.C., Quesada M. and Wınsor J.A. 1992. Factors that influence pollen performance In: Ecology and Evolution of Plant Reproduction (R. Wyatt, ed,) pp.119-134

Tosun (Güçlü), F. and Koyuncu F., 2007. Investigations of suitable pollinator for 0900 Ziraat sweet cherry cv.: pollen performance tests, germination tests, germination procedures, *in vitro* and *in vivo* pollinations. HortSci(Prague), 34, (2), 47-53

Travers S.E., 1999. Environmental effects on components of pollen performance in *Faramea occidentalis* (L.) A. Rich. (*Rubiaceae*). Biotropica, 31 (1), 159-166.

Vasılakakıs M. and. Porlıngıs I.C, 1985. Effect of temperature on pollen germination, pollen tube growth, effective pollination period, and fruit set of pear. Hortsci., 20, 733-735.

Zınn E.K., Tunç-Özdemır M., and Harper F.J., 2010. Temperature stress and plant sexual reproduction: uncovering the weakest links. J Exp Bot 61 (7), 1959–1968.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE EFFECTS OF DIFFERENT GROWING SYSTEMS ON THE YIELD AND QUALITY OF CURRANT CULTIVATION

Volkan OKATAN[1], Mehmet Atilla AŞKIN[2]

[1]Usak University, Sivasli Vocational School, Usak, Turkey
[2]Suleyman Demirel University, Agricultural Faculty, Department of Horticulture, Isparta, Turkey

Corresponding author email: okatan.volkan@gmail.com

Abstract

In this study, two different currants varieties ('Rosenthal' and 'Red Lake') were investigated. The row spacing, number of branches and pruning practices has been evaluated in terms of their effects on yield and quality of the varieties. For this purpose, during three-year period (2014-2016) it were made three applications to black currant: the distance between rows and plants in the row 2.0 x 1.2 m, 2.0 x 1.5 m and 2.0 x 2.0 m, training bushes as 1, 3 and 5 branches per plants. In this application as well as other parts of plants, the pruning performed by keeping a part allowed pruning old shoots. Different biochemical, phenological and pomological properties have been evaluated too.

Key words: *currant, 'Rosenthal', 'Red Lake', fruit, yield, Uşak.*

INTRODUCTION

Many of the plant species used as berries are naturally grown in Turkey. These berries are rich in vitamins and minerals, also important for human health and their use in the food sector is increasing (Karaer and Adak, 2006). One of the well-known families with berries is Grossulariaceae.

The leaves of the currants are round, 3-5 slices, quite broad (about 12 cm) and have short shoots. The flowers are greenish, greenish-brown. The male organs are shorter than the bowl leaves all around. The bowl leaves are red-pointed and bowl shaped. Flowering in our country (Turkey) corresponds to April-May. The plant can reach up to 2 m in height (Islam, 2010).

The flower buds of the red currants are longer than the flower buds of the black currants. Bees or other insects are the main pollinators of currants. The pollen of most blackcurrants is spread from anthers between 2:00 and 6:00 am, so most pollination occurs during the day.

Currants are rich in organic matter, have a high water holding capacity, are well ventilated and grow well in soil with a pH ranging between 5.5 to 7.0 (Hummer and Dale, 2010). Choosing a place to grow a healthy plant is one of the most important factors. For good yield, plants should be planted in good sunlight areas. Cultivation techniques, such as fertilization and irrigation, must be performed in the canopy areas, on the northern slopes of the land, or in higher altitude areas (Barney and Hummer, 2005).

The natural value for the production of currant juice, fruit juice and other beverage products is considered high raw material. Fruits are preferred because of their sensory qualities such as color, taste and taste (Píry et al., 1995; Brennan et al., 2003).

The berry is also suitable for freezing and storage at the same time. In addition, it is made from fruit concentrates; jams, pastries and pies, ice cream, flavored mineral water and sugar for children. Cream, liqueur and white wine are made from the fruits in various countries (Brennan, 1996). In Sweden, 40% of the currant's production is used in the production of vodka and local alcoholic beverages, most of which is fresh on the market (Brennan, 2008).

The objective of this study is to establish a closed garden as an alternative fruit of nursery growing naturally in our country, to carry out different cultivation practices and to find the most suitable planting system.

MATERIALS AND METHODS

This study was carried out in a private enterprise farm in Sivaslı-Usak between 2014-2016. The altitude of the experimental area is

1044 m and the coordinates are 38° 29' 15.75' 'North and 29° 42' 16.53 " East. Soil samples were taken from 0-30 and 30-60 cm depths on 15.03.2014 for the physical and chemical analyzes of the test soil.

Soil analysis was carried out at the laboratory of Suleyman Demirel University, Faculty of Agriculture, Soil Department. The analysis results are given in Table 1.

In the study, one year old plants of 'Red Lake' (red currant variety) and 'Rosenthal' (black currant variety) were used. Currants were planted on April 15, 2014 in accordance with the design of the trial. Trial 3 was set up as a repeater.

In each experimental plot, 9 plants were evaluated and 3 plants were evaluated as 1 replicate.

Table 1. Physical and chemical soil properties of the trial area (2014)

Soil properties		Soil depth		Evaluation
		0-30 cm	30-60 cm	
Physical analysis	Sand (%)	67	72	Sandy loamy
	Plate (%)	19	12	
	Clay (%)	14	16	
	Salt (mmhos/cm)	140.8	129.8	Without salt
	pH	7.95	7.94	Light alkali
	Lime (%)	34.23	39.9	
Chemical analysis	Organic matter (%)	2.28	1.68	
	Nitrogen (ppm)	1142	839.68	
	Phosphorus (ppm)	8.97	0.79	
	Potassium (ppm)	128.4	82.2	
	Calcium (ppm)	4078.6	2453.4	
	Magnesium (ppm)	60.94	39.16	
	Iron (ppm)	2.74	2.77	
	Copper (ppm)	0.45	0.44	
	Mangan (ppm)	4.75	4.13	
	Zinc (ppm)	0.47	0.29	

The first objective of the experiment is the evaluation of the effect on the yield and quality of the different spacing distance. For this purpose, the varieties were planted on 15.04.2014 as 2 m x 2 m, 1.5 m x 2 m and 1.2 m x 2 m modules. In the same day, the drip irrigation system was installed in the trial area. Another aspect of the experiment was directed to the effect on yield and quality of different number of branches per plant: 1, 3 and 5. The last approach of the experiment was the effect of pruning on the yield and quality of the currant fruits. In this regard, the old branches of the plants were pruned and the new branches bear fruits, while the young shoots of the other half were pruned and the fruits were picked from the old branches.

In terms of plant characteristics of the varieties of currants; Shoot diameter (mm) and shoot length (mm), in terms of pomological characteristics; Fruit weight (g), fruit width (mm), fruit length (mm) and yield per plant (g) and some biochemical properties; Soluble solid content (SSC) (%) (%), pH (%) and Titratable acidity (TA) (%) were examined.

RESULTS AND DISCUSSIONS

The diameter and length of the shoots for both varieties are presented in Table 2.

According to the measurements, the maximum shoot diameter in the 'Rosenthal' variety was found to be at 2.0×1.5 m in the planting interval and in the three branches without pruning system as 7.28 mm in average, and in the 'Red Lake' variety was found at 2.0×2.0 m planting interval area and in the three branches without pruning system as 7.40 mm in average.

The shoot length of 'Rosenthal' variety was found to be 2.0×1.5 m in the planting interval and in the single branches with pruning system as 63.02 mm in average, and in the 'Red Lake' variety was found at 2.0×1.5 m planting interval area and in the single branch without pruning system as 44.47 mm in average.

Table 2. Averages of shoot diameters and shoot lengths of varieties in the years 2015-2016

Pruning	Distance (m)	Number of branches	Shoot diameter (mm)		Shoot length (mm)	
			Rosenthal	Red Lake	Rosenthal	Red Lake
Applied	2x1.2	1	6.80±0.12	6.98±0.82	47.29±2.17	42.65±4.85
		3	6.62±0.26	5.87±0.15	49.27±2.13	46.56±6.14
		5	2.48±0.17	6.33±0.26	36.94±3.27	36.77±3.73
	2x1.5	1	6.47±0.63	6.74±0.73	63.02±5.82	41.85±4.15
		3	4.40±0.05	6.63±0.12	46.25±4.16	37.05±2.15
		5	5.76±0.27	6.59±0.48	40.98±6.02	33.59±6.41
	2x2.0	1	6.29±0.13	7.09±0.32	26.55±3.45	44.25±3.25
		3	5.83±0.18	6.56±0.39	32.82±4.88	34.72±7.08
		5	5.95±0.44	6.16±0.11	27.27±3.93	24.77±2.75
Non-Applied	2x1.2	1	6.69±0.31	7.08±0.22	40.47±2.73	38.74±3
		3	6.63±0.17	6.09±0.91	45.90±6.10	37.39±18
		5	6.01±0.19	5.66±0.02	34.60±4.18	28.41±2
	2x1.5	1	6.41±0.12	6.76±1.24	45.10±2.27	44.47±47
		3	7.28±0.83	7.07±0.33	47.33±5.13	43.48±3.46
		5	6.22±0.65	6.67±0.73	43.19±4.19	30.62±1.15
	2x2.0	1	5.98±0.43	6.67±0.26	44.95±7.15	32.68±4.21
		3	6.45±0.71	7.40±0.50	42.97±8.11	42.79±4.65
		5	5.29±0.09	6.47±0.83	24.96±4.93	20.11±2.17

Djordjević et al. (2014) reported an average shoot diameter of 6.5 to 15.1 mm in a study of currants in Belgrade. In another study conducted in Belgrade, it was reported that the average shoot diameters in currants are between 4.73 and 11.60 mm (Milivojević et al., 2010).

The number of bunch, fruit number in bunch and bunch length belonging to the applications in varieties are presented in Table 3.

According to the measurements, the maximum number of bunch in the 'Rosenthal' variety was found to be 2.0×1.2 m in the planting interval and in the five branches without pruning system as 18.84 in average, and maximum number of bunch in the 'Red Lake' variety was found at 2.0×1.5 m planting interval area and in the three branches with pruning system as 56.85 in average. The maximum fruit number of bunch in the 'Rosenthal' variety was found to be 2.0×2.0 m in the planting interval and in the three branches without pruning system as 12.5 in average, the maximum fruit number of bunch in the 'Red Lake' variety was found at 2.0×2.0 m planting interval area and in the three branches with pruning system as 30.6 in average. The maximum bunch length in the 'Rosenthal' variety was found to be 2.0×2.0 m in the planting interval and in the five branches with pruning system as 30.6 in average, the maximum bunch length in the 'Red Lake' variety was found at 2.0×2.0 m planting interval area and in the three branches without pruning system as 94.24 in average.

In a study conducted in Trabzon, it was examined the number of bunches in the currants and the most fruit bunches had 'Jonkheer van Tets' (78.10) and the least fruit bunches are 'Ojebyn' (27.20) and 'Detvan' (25.20) (Çelik, 2012). In an adaptation study conducted in Tokat ecology, it was reported that the number of bunch varieties varied between 1.78-2.47 in one branch (Gerçekçioğlu et al., 2009).

Fruit weight (g), fruit width (mm), fruit length (mm) and yield per plant (g) belonging to the measurements of varieties are presented in Table 4.

Table 3. Number of bunches, number of fruits and average length of bunches in the years 2015-2016 for experimented currant varieties

Pruning	Distance (m)	Number of branches	Number of bunch		Fruit number of bunch		Bunch lenght (mm)	
			Rosenthal	Red Lake	Rosenthal	Red Lake	Rosenthal	Red Lake
Applied	2x1.2	1	5.67±1.25	18.31±2.83	9.90±2.74	28.00±3.16	43.39±4.15	67.65±8.95
		3	10.34±2.48	13.50±1.95	10.00±2.00	18.65±2.74	43.26±5.18	49.85±6.16
		5	18.84±3.16	34.12±3.18	8.65±1.89	30.15±2.18	39.22±4.29	85.94±5.28
	2x1.5	1	5.34±2.18	14.34±1.83	10.10±2.74	26.80±4.86	48.87±3.95	51.30±5.12
		3	11.83±1.78	52.54±5.81	10.20±3.15	28.45±3.26	41.18±3.87	71.01±6.92
		5	17.18±1.45	50.32±4.72	9.35±2.73	28.85±3.16	36.94±5.91	92.67±8.16
	2x2.0	1	4.67±1.90	19.83±3.91	11.23±3.18	26.20±3.91	48.17±2.18	88.45±7.59
		3	10.15±1.25	53.65±4.17	12.15±4.90	27.90±2.85	58.35±4.19	94.24±9.28
		5	19.70±2.15	43.34±4.18	9.04±8.76	28.65±2.45	42.70±6.13	87.38±7.16
Non-Applied	2x1.2	1	5.00±1.00	17.67±3.50	10.40±2.60	29.65±2.67	43.22±5.80	68.79±7.23
		3	15.01±2.00	45.84±7.82	11.72±3.28	28.45±3.16	42.24±2.75	69.63±8.16
		5	18.5±3.48	34.15±9.18	11.30±2.17	29.45±3.71	56.52±7.19	91.57±9.11
	2x1.5	1	5.17±1.82	14.84±5.17	11.60±2.82	28.10±4.85	42.50±8.20	89.47±10.93
		3	12.23±1.39	56.85±12.23	12.30±2.91	30.05±3.76	43.00±4.75	68.67±2.90
		5	17.69±1.82	46.00±4.00	11.61±2.35	29.00±4.00	57.27±9.18	86.53±5.17
	2x2.0	1	8.00±1.50	26.17±8.12	11.20±2.41	29.35±1.91	62.40±6.21	87.56±4.18
		3	9.34±1.23	42.81±3.64	11.75±1.86	30.60±4.18	42.87±4.27	69.50±3.17
		5	15.00±2.08	49.17±5.82	11.05±1.18	27.10±3.19	58.64±6.13	50.69±5.16

Table 4. Average of fruit weight, fruit length and yield per plant for currant varieties (2015-2016)

Pruning	Distance (m)	Number of branches	Fruit weight (g)		Fruit width (mm)		Fruit length (mm)		Yield per plant (g)	
			Rosenthal	Red Lake	Rosenthal	Red Lake	Rosenthal	Red Lake	Rosenthal	Red Lake
Applied	2x1.2	1	0.59±0.02	0.37±0.05	9.91±0.76	8.27±0.22	9.79±0.62	7.77±0.82	32.23±4.16	191.27±15.18
		3	0.70±0.05	0.54±0.02	9.88±0.92	9.54±0.38	9.76±0.54	10.05±0.93	70.47±12.75	173.33±12.17
		5	0.92±0.03	0.46±0.04	9.56±0.62	9.35±0.17	10.16±0.76	8.50±0.54	95.19±14.83	475.13±11.18
	2x1.5	1	0.64±0.05	0.56±0.05	11.16±0.48	9.82±0.26	11.02±0.17	10.34±0.87	35.23±12.18	210.54±27.83
		3	0.54±0.05	0.38±0.02	9.66±0.27	8.74±0.31	9.38±0.98	7.85±0.81	65.34±11.00	571.58±47.20
		5	0.91±0.07	0.55±0.07	12.62±0.18	9.91±0.24	9.88±0.32	9.00±0.18	217.28±19.91	801.62±56.18
	2x2.0	1	0.85±0.05	0.47±0.08	11.31±0.82	9.63±0.92	11.49±1.32	8.75±0.21	40.47±12.83	249.84±14.84
		3	0.40±0.08	0.55±0.06	10.17±0.26	10.08±0.21	8.62±0.87	9.15±0.46	25.54±16.23	817.60±25.19
		5	0.65±0.04	0.47±0.05	9.98±0.46	9.47±0.85	9.63±0.46	8.64±0.89	119.51±13.07	572.83±38.69
Non-Applied	2x1.2	1	0.59±0.06	0.38±0.05	9.87±0.81	8.37±0.18	9.75±0.54	7.90±0.08	30.28±8.15	198.52±26.51
		3	1.23±0.07	0.38±0.04	12.72±0.73	8.51±1.23	12.59±0.39	8.00±0.20	198.92±15.82	483.63±22.94
		5	1.18±0.09	0.54±0.09	12.66±0.19	9.79±0.73	12.72±0.76	8.89±0.19	230.33±26.92	544.41±32.82
	2x1.5	1	0.56±0.08	0.43±0.01	9.70±0.28	8.88±0.86	9.62±0.65	8.28±0.58	34.70±7.16	179.48±18.94
		3	0.57±0.05	0.37±0.00	9.82±0.10	8.49±0.81	9.70±0.20	7.59±0.17	85.46±8.12	618.51±14.72
		5	1.05±0.01	0.41±0.12	12.36±0.93	8.63±0.37	11.52±0.03	8.01±0.20	185.11±22.85	554.39±16.90
	2x2.0	1	0.28±0.02	0.42±0.08	7.62±0.17	8.73±0.42	7.41±0.19	8.10±0.15	24.32±5.19	319.94±18.23
		3	0.57±0.03	0.37±0.04	9.88±0.26	8.60±0.29	9.76±0.05	7.69±0.09	62.82±16.12	474.99±10.82
		5	0.72±0.08	0.55±0.15	10.52±0.82	9.66±0.82	10.27±1.12	10.22±0.14	110.59±11.10	783.13±19.95

According to the measurements, the maximum fruit weight in the 'Rosenthal 'variety was found to be 2.0×1.2 m in the planting interval and in the three branches without pruning system as 1.23 g in average, and in the 'Red Lake' variety was found at 2.0×1.5 m planting interval area and in the single branches with pruning system as 50.56 g in average. The maximum fruit width in the 'Rosenthal' variety was found to be 2.0×1.2 m in the planting interval and in the three branches without pruning system as 12.72 mm in average, and in the 'Red Lake' variety was found at 2.0×1.2 m planting interval area and in the three branches with pruning system as 10.08 mm in average. The maximum fruit width in the 'Rosenthal' variety was found to be 2.0×1.2 m in the planting interval and in the five branches without pruning system as 12.72 mm in average, and in the 'Red Lake' variety was found at 2.0×1.5 m planting interval area and in the single branch with pruning system as 10.34 mm in average. The maximum yield of per plant in the 'Rosenthal' variety was found to be 2.0×1.2 m in the planting interval and in the five branches without pruning system as 12.72 mm in average, and in the 'Red Lake' variety was found at 2.0×1.5 m planting interval area and in the single branch with pruning system as 10.34 mm in average. The maximum yield of per plant in the 'Rosenthal' variety was found to be 2.0×1.2 m in the planting interval and in the five branches without pruning system as 230.33 g in average, and in the 'Red Lake' variety was found at 2.0×1.5 m planting interval area and in the five branches with pruning system as 801.62 g in average.

In a study conducted by Nikolic et al. (2006), pomological characters of currant varieties to determine the fruit weight of varieties 0.90-2.36 g. In an adaptation study conducted in Samsun ecology, it was reported that the fruit width of currants varieties varied between 9.56-14.10 mm (Kaplan and Akbulut, 2006). Gerçekçioğlu et al. (2009), in their study of Tokat conditions, they determined that fruit length of currants varieties changed between 8.01 and 13.99 mm. Göktaş et al. (2006), in their study in Isparta Eğirdir region, the highest yield per plant in the varieties of currants Tokat 3 (4802,59 g), while the lowest yield was found in Tokat 2 (422.27 g).

Soluble solid content (%), pH and Titratable acidity (%) values are presented in Table 5.

Table 5. Average of SSC, pH and TA values of currant varieties (2015-2016)

Pruning	Distance (m)	Number of branches	Soluble solid content (%)		pH		Titratable acidity (%)	
			Rosenthal	Red Lake	Rosenthal	Red Lake	Rosenthal	Red Lake
Applied	2x1.2	1	14.64±0.66	12.25±1.63	2.69±0.36	2.77±0.03	4.02±0.24	2.85±0.62
		3	14.19±0.54	12.40±0.87	2.55±0.24	3.08±0.41	3.53±0.01	2.87±0.21
		5	14.19±0.11	12.07±0.82	2.66±0.47	2.61±0.08	3.52±0.05	2.97±0.06
	2x1.5	1	14.72±0.36	11.82±0.45	2.71±0.84	2.62±0.02	3.30±0.19	3.28±0.79
		3	13.52±0.87	11.68±0.58	2.60±0.22	2.84±0.05	3.99±0.12	2.87±0.47
		5	15.58±0.79	11.27±0.29	2.64±0.17	2.84±0.01	3.62±0.06	2.95±0.18
	2x2.0	1	15.30±0.18	13.00±0.37	2.76±0.35	2.92±0.04	3.31±0.05	2.70±0.00
		3	13.44±0.12	12.64±0.18	2.36±0.20	2.64±0.00	3.33±0.62	2.90±0.05
		5	14.20±0.36	11.57±0.99	2.61±0.12	2.83±0.17	3.83±0.21	2.78±0.05
Non-Applied	2x1.2	1	15.44±0.44	12.55±0.25	2.68±0.18	3.04±0.13	3.31±0.00	3.34±0.06
		3	14.42±0.28	11.65±1.13	2.55±0.17	2.86±0.06	4.09±0.14	2.72±0.17
		5	14.02±0.91	12.07±0.72	2.72±0.36	2.83±0.22	3.56±0.27	2.90±0.08
	2x1.5	1	15.47±0.53	12.05±0.14	2.64±0.74	2.73±0.17	3.56±0.32	2.90±0.15
		3	14.13±0.12	12.07±0.38	2.43±0.49	2.73±0.16	3.49±0.33	2.70±0.01
		5	14.87±0.23	11.63±0.12	2.62±0.57	2.98±0.62	3.55±0.17	2.84±0.11
	2x2.0	1	15.37±0.57	11.50±0.63	2.68±0.15	2.97±0.18	3.48±0.12	2.69±0.04
		3	15.80±0.11	11.69±0.14	2.55±0.22	2.95±0.24	3.40±0.05	3.26±0.17
		5	14.57±0.26	12.27±0.13	2.95±0.46	2.79±0.26	3.65±0.15	3.13±0.22

According to the measurements, the maximum SSC in the 'Rosenthal' variety was found to be 2.0×2.0 m in the planting interval and in the three branches without pruning system as 15.80 % in average, and in the 'Red Lake' variety was found at 2.0×2.0 m planting interval area and in the single branches with pruning system as 13.00 % in average. The maximum pH in the 'Rosenthal' variety was found to be 2.0×2.0 m in the planting interval and in the five branches without pruning system as 2.95 % in average, and in the 'Red Lake' variety was found at 2.0×1.2 m planting interval area and in the three branches with pruning system as 3.08 % in average.

The highest TA of 'Rosenthal' was found to be 2.0×1.2 m in the planting interval and in the singel branch with pruning system as 4.02 % in average, and in the 'Red Lake' variety was found at 2.0×1.2 m planting interval area and in the single branch without pruning system as 3.34 % in average.

Kaplan and Akbulut (2006), in their study of Samsun Çarşamba Region, they determined that SSC of black currants varieties changed between 14.83-15.53 % and red currants varieties changed 9.26-10.43 %.

Zatylny et al. (2004), their study of Saskatchewan province in Canadian, the chemical of the fruits of currants varieties. According to the obtained results, PH value 2.85-3.04; Titration acidity values of 3.04-4.03% and briks Values are in the range of 15.1-16.6 %. The findings of our findings are similar to those of other investigators.

CONCLUSIONS

In terms of production, 'Rosenthal' variety performed better at 2.0×1.2 m distance, 5 branches with pruning system (average yield of 96 kg per/decar), and for the 'Red Lake' variety 2.0×2.0 m planting distance 3 branches and without pruning system (average yield of 205 kg per/decar).

ACKNOWLEDGEMENTS

This PhD study was supported by Süleyman Demirel University Scientific Research Projects Administration Unit with Project No: 4510-D1-15.

REFERENCES

Barney D.L. ve Hummer K.E., 2005. Currants, Gooseberries, and Jostaberries: A Guide For Growers, Marketers, and Researchers in North America. New York: Haworth Press Inc. 260 p.

Brennan R.M. (1996). Currants and Gooseberries. Chapter 3 pp. 191-295 in: J. Janick and J. N. Moore (eds.) Fruit Breeding, Vol. II Vine and Small Fruit Crops. John Wiley & Sons. Inc. N.Y.

Brennan A.C., Harris S.A., Hiscock S.J., 2003. The population genetics of sporophytic self–incompatibility in *Senecio squalidus* L. (*Asteraceae*): avoidance of mating constraints imposed by low S–allele number.Philosophical Transactions of the Royal Society B: Biological Sciences,358(1434), 1047-1050.

Brennan R.M., 2008. Currants and Gooseberries, Temperate Fruit Crop Breeding, J.F. Hancock (ed.), Chapter 6, Scotland, UK. 177-196,

Çelik E., 2012. Organik olarak yetiştirilen frenküzümü ve bektaşiüzümü çeşitlerinin bazı özelliklerinin araştırılması. Y.Lisans Tezi.

Djordjević B., Rakonjac V., Akšić M. F., Šavikin K., Vulić T., 2014. Pomological and biochemical characterization of European currant berry (*Ribes* spp.) cultivars. Scientia Horticulturae, 165, 156-162.

Gerçekçioğlu R., Bayazıt S., Edizer Y., Çekic Ç., 2009. Bazı Frenküzümü (*Ribes* ssp.) Çeşitlerinin Tokat Ekolojisindeki Performansları. III. Ulusal Üzümsü Meyveler Sempozyumu, 308-313, Kahramanmaraş.

Göktaş A., Demirtaş İ., Atasay A., 2006. Bazı Böğürtlen ve Frenküzümü Çeşitlerinin Eğirdir (Isparta) Yöresine Adaptasyonu. II. Ulusal Üzümsü Meyveler Sempozyumu, s.151-156, Tokat.

Hummer K.E., Dale A., 2010. Horticulture of Ribes. Forest Pathology, 40, p.251- 263.

İslam A., 2010. Üzümsü Meyve Yetiştiriciliği Lisans Ders Notu, Ordu Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü, 42 s.

Kaplan N., Akbulut M., 2006. Samsun Çarşamba Ovası Koşularına Uygun Frenküzümü Çeşitlerinin Belirlenmesi. II. Ulusal Üzümsü Meyveler Sempozyumu, s.145-150, Tokat.

Karaer F., Adak Y., 2006. Türkiye florasında üzümsü meyve olarak kullanılan taksonların yayılış alanları ve ekolojik özellikleri. II. Ulusal Üzümsü Meyveler Sempozyumu, Bildiriler: 36-43, 14-16 Eylül 2006/Tokat.

Milivojević J., Bogdanović-Pristov J., Maksimović V., 2010. Phenolic compounds and vitamin C as sources of antioxidant activity in black currant fruit (*Ribes nigrum* L.). Acta Agriculturae Serbica, 15(29), 3-10.

Nikolic M., Vulic T., Milivojevic J., Dordevic B., 2006. Pomological Characteristics of Newly Introduced Black Currant Cultivars (*Ribes nigrum* L.). International Conference of Perspectives in Europen Fruit 185 Growing. (Editör: Dipl. Ing. T. Necas, Ph. D.), Basımevi MZLU in Brno, 150. Baskı, s: 200-203, Lednice, Czech Republic.

Píry J., Príbela A., Ďurčanská J., Farkaš P., 1995. Fractionation of volatiles from blackcurrant (*Ribes nigrum* L.) by different extractive methods.Food chemistry, 54(1), 73-77.

Zatylny A.M., Ziehl W.D., ve St-Pierre R.G., 2004. Physicochemical Properties of Fruit of Chokecherry (*Prunus virginiana* L.), Highbush Cranberry (*Viburnum trilobum* Marsh.), and Black Currant (*Ribes nigrum* L.) Cultivars Grown in Saskatchewan. Canadian Journal of Plant Science Fruit Physicochemical Properties, 425-429.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPARATIVE STUDY OF PROCESSED PRODUCTS FROM CULTIVARS OF THE NATIVE APRICOT

Constanța ALEXE[1], Marian VINTILĂ[1], Ion CAPLAN[2], Gheorghe LĂMUREANU[2], Lenuța CHIRA[3]

[1]Research and Development Institute for Processing and Marketing of the Horticultural Products - Bucharest, No. 1A, Intrarea Binelui Street, District 4, 042159, Bucharest, Romania
[2]Research Station for Fruit Growing (RSFG) Constanta, 1 Pepinierei Street, 907300, Commune Valu lui Traian, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, 011464, Bucharest, Romania

Corresponding author email: tantialexe@yahoo.com

Abstract

The paper aimed to present the suitability of processing the seven native apricot cultivars grown at the Research Station for Fruit Growing Constanta: 'Tudor', 'Sirena', 'Orizont', 'Olimp', 'Neptun', 'Augustin' and 'Litoral', results leading to the establishment of the fruit valorization direction. Apricots were processed at the Research and Development Institute for Processing and Marketing of the Horticultural Products - Bucharest (micro lab) as compote, jam, comfiture and nectar. The cans'quality assessment was performed using Method A - STAS 12656-8, the state standard that regulates the analysis methods with unitary score scales, used to evaluate the organoleptic characteristics of food. The results show that these cultivars have in common a sweet, pleasant, aromatic flavor (which is why they are highly appreciated for fresh consumption), characteristics that, at the same time, influence the quality of processed products. Out of the studied cultivars, 'Olimp' was particularly highlighted, which is very well suitable to all processing into four types of canned analyzed: comfiture, jam, compote and nectar, the resulting product having remarkable sensory qualities. Apricots in the 'Tudor' cultivars are less suitable for processing, preferably as being able to consume as fresh fruits or, possibly, as comfiture or nectar. In conclusion: for getting canned compote, the 'Orizont', 'Olimp', 'Neptun' cultivars are recommended; for comfiture, cultivars 'Tudor', 'Olimp', 'Augustin', 'Litoral'; 'Orizont', 'Neptun', 'Litoral' cultivars for jam; for nectar, the 'Sirena', 'Orizont', 'Olimp', 'Neptun' cultivars.

Key words: *compote, comfiture, jam, nectar, quality.*

INTRODUCTION

Apricots are very popular with consumers, both as a dessert fruit and as well as processed in various ways.

The high demand for fruits is determined by their qualitative and technological attributes, by the complex biochemical composition and by the very pleasant taste and specific flavor etc.

There are many apricot consumption benefits that are also supported by scientific studies.

Firstly, they are a real and rich source of vitamin A, B and C, along with beta carotene (due to which the color is yellow-orange) helps maintain eyesight and nerves and tissue regeneration (www.pro-sanatate.com/caisele-beneficii...*)*.

But in the biochemical composition of fruits there are several other important components for the human nutrition, including: 10.6 to 21.7% dry substance, 6 to 16.6% total sugar, 0.55 to 1.1% pectin , 1.09 to 1.64% protein, 0.6 to 0.86% minerals out of which: K 75-112 mg%, P 21-32 mg% 6-14 mg% Ca, mg, S, Na , 0.41 to 3.20 mg%, vitamin P, 0.72 to 1.8 mg% vitamin E, and the energy value is 21-77 calories per 100 g etc. As shown by the above data, through the biochemical composition, apricots ensure all components the human body needs to conduct metabolism in good condition. Apricots are used for their favorable effect on digestion due to alkaline reaction, in the production of hemoglobin in anemia etc. High nutritional value of apricots and apricot-based finished products, led specialists in the scientific research domain to diversify the assortment by creating or placing cultivars in the tillage that behave well in the climatic conditions from Romania. Because apricots are not suitable for fresh storage more than a short period of time, processing them as canned represents a needed and desired solution.

But fruit conservation suitability is a cultivar characteristic; therefore, studies on the potential of different cultivars to be processed in one form or another are necessary.

Lately, in our country, there have been many concerns in this direction for the species: cherries, peaches, sweet cherries (Caplan et al., 2015; Caplan et al., 2016; Lamureanu et al., 2014; Lamureanu et al. 2015; Sarbu et al., 2010; Veringa et Dumitrescu, 2016; Vintila et al., 2015).

When manufacturing, consuming and evaluating the quality of processed products, we must consider the provisions of state or professional standards that regulate quality technical conditions of raw and auxiliary materials, the organoleptic and physicochemical properties of the finished product etc.

In the present work, which final aims to establish the destination of improvement by setting the processing suitability of cultivars of apricot, these standards were taken into consideration: STAS 3164-90. Fruit compote. Standard State; STAS 3750-90. Comfiture. Standard State; STAS 3183-90. Jams. Standard State; SP 877-96. Fruit nectar. Standard Professional.

MATERIALS AND METHODS

During 2015-2016 studies and research were carried out, aiming at determining the suitability of processing of seven local native cultivars of apricots that exist in culture at Research Station for Fruit Growing Constanta: ‘Tudor’, ‘Sirena’, ‘Orizont’, ‘Olimp’, ‘Neptun’, ‘Augustin’ and ‘Litoral’.

For this purpose apricots were processed as compote with whole fruits, comfiture, jam and nectar at the Research and Development Institute for Industrialization and Marketing of Horticultural Products in Bucharest, in the micro-production lab._After the period required to stabilize the product (21 days), the cans were subjected to sensory analysis, applying the evaluation method A- STAS 12656-88, which establishes the me-thods of analysis using unitary score scales (used in evaluating the organoleptic characteristics of food).

This method is applied in order to assess a combination of organoleptic properties: appearance, color, taste, texture or, where appropriate, consistency.

Each organoleptic evaluation was made by comparing with the unitary score scales from 0-5 points and got the average score given by the group of tasters, based on individual sheets of recording the marks that were given.

The score of weighted average was calculated, adding these for obtaining the overall average score and the organoleptic qualities of the products on the basis of total average, by comparison with a scale of 0 - 20 points were settled.

Finally they awarded qualifiers for each product and cultivar. In the overall score achieved by the various analyzed products, we differentiate between 5 quality classes: very good (18.1-20.0), good (15.1-18.0), satisfactory (11.1-15.0), unsatisfactory (7.1-11.0) and incompatible (0-7.0).

Before processing, fresh fruit of every cultivar were organoleptic analyzed and characterized in terms of size, shape, color and flesh peeling, texture, taste and aroma pulp, the kernel size etc.

It has also been tested and shown resistance to keeping temporary apricots of every cultivar.

RESULTS AND DISCUSSIONS

The fruit from the **‘Tudor’** cultivar has a good resistance for keeping fresh, is medium-sized, averaging 40-45 g, ovoid shaped, slightly dorsal-ventrally flattened with orange peel covered with carmine red on 2/3.

The flesh is orange, non-adherent to the kernel, juicy, fragrant, and the kernel is large with bitter core.

The sensory analysis of processed products (Table 1) highlights the fact that, according to the product, their quality differs greatly from ”satisfactory” in the case of compote and jam, to ”very good” in the case of comfiture.

In the Figure 1, we have the deliverables: comfiture, nectar, compote and apricot jam from the ‘Tudor’ cultivar.

The **‘Sirena’** cultivar fruit is medium to large on average 65 g, globular to ovoid shape, slightly asymmetrical, with good resistance to fresh storage.

The peel is orange with red spots on the sunny side. The orange pulp has a sturdy structure, juicy enough, pleasant taste and fine flavor.
The stone represents 6.1% of the weight of the fruit and has a sweet core.

Table 1. Sensory analysis of the apricot processed products from the 'Tudor' cultivar

MU=points

Specification	Product			
	Compote	Comfiture	Jam	Nectar
Aspect	4.08	5.72	4.80	3.84
Color	3.04	5.32	3.68	2.72
Taste	4.08	3.72	3.84	5.76
Consistency	3.04	3.72	2.40	4.00
Overall average score	14.24	18.48	14.72	16.32
Qualificative	satisfactory	very good	satisfactory	good

Fig 1. Apricot processed products from the 'Tudor' cultivar

Out of the four types of processed products (Fig. 2), the nectar is highlighted with 18.24 points and the qualification ''very good''.
The compote, with only 14.25 points scored ''satisfactory'' (Table 2).

Table 2. Sensory analysis of the apricot processed products from the 'Sirena' cultivar

MU=points

Specification	Product			
	Compote	Comfiture	Jam	Nectar
Aspect	3.81	5.06	4.56	5.12
Color	3.77	5.06	2.56	3.68
Taste	4.00	3.55	5.04	5.76
Consistency	2.67	3.55	3.36	3.68
Overall average score	14.25	17.22	15.52	18.24
Qualificative	satisfactory	good	good	very good

For the comfiture (17.22 points) and the jam (15.52 points) the qualificative that was awarded was ''good''.

Fig. 2. Apricot processed products from the 'Sirena' cultivar

The **'Orizont'** variety, with good resistance to fresh storage has an oblong shaped fruit, medium to large weight (45.8 to 62.3 g).
The peel is orange, with carmine red on the sunny side. The flesh is orange; fine textured, of an average firmness, aromatic, very juicy.
The kernel is medium-sized, oblong shaped, adherent to the flesh, with sweet core. The data in Table 3 shows a good suitability at processing to 'Orizont' cultivar, compote (18.32 points), nectar (18.16 points) getting a''very good'' qualificative, comfiture (17.22 points) and jam (15.84 points) the''good'' qualificative.

Table 3. Sensory analysis of the apricot processed products from the 'Orizont' cultivar

MU=points

Specification	Product			
	Compote	Comfiture	Jam	Nectar
Aspect	4.80	5.32	4,32	5,.6
Color	3.52	4.86	2.72	3.20
Taste	6.00	3.49	5.76	5.52
Consistency	4.00	3.55	3.04	3.68
Overall average score	18.32	17.22	15.84	18.16
Qualificative	very good	good	good	very good

The four types of products obtained from the 'Orizont' cultivar are presented in Figure 3.
The **'Olimp'** cultivar has good resistance to preserve the fruit in fresh state. The fruit is big (65-75g) with orange skins and the flesh is bright orange, with firm texture, good flavor, very good and balanced taste. All four types obtained by processing received very high

marks from tasters, which lead to the unique qualificative of "very good" (Table 4 and Figure 4).

Fig. 3. Apricot processed products from the 'Orizont' cultivar

Table 4. Sensory analysis of the apricot processed products from the 'Olimp' cultivar

MU=points

Specification	Product			
	Compote	Comfiture	Jam	Nectar
Aspect	5.04	6.00	6.00	5,36
Color	3.36	3.20	5.32	3.84
Taste	6.00	6.00	3.90	5.76
Consistency	4.00	3.68	4.00	3.52
Overall average score	18.40	18.88	19.22	18.48
Qualificative	very good	very good	very good	very good

The compote has obtained the maximum score for taste and consistency, the comfiture for aspect and taste and the jam for aspect and consistency.

The **'Neptun'** cultivar, with good resistance for fresh storage, has a large fruit, ovoid, sharper at the top, yellow, striped and red dotted on the sunny side. The flesh is yellow-orange, firm, sweet and slightly fizzy, appreciated for meal.

Figure 4. Apricot procesed products from the 'Olimp' cultivar

The canned version is also praised (Table 5 and Figure 5), given that outside of sweetness, which received a "good" qualificative, the other assortments were employed by tasters in the "very good" column.

Table 5. Sensory analysis of the apricot processed products from the 'Neptun' cultivar

MU=points

Specification	Product			
	Compote	Comfiture	Jam	Nectar
Aspect	5.76	5.72	6.00	6.00
Color	3.36	4.39	6,00	4.00
Taste	6.00	3.46	3.77	6.00
Consistency	3.52	3.81	4.00	3.52
Overall average score	18.64	17.38	19.77	19.52
Qualificative	very good	good	very good	very good

The jam (19.77 points) as well as the nectar (19.52 points) has received score close to the highest.

Figure 5. Apricot processed products from the 'Neptun' cultivar

The **'Augustin'** cultivar shows cordiforme fruit shape, with average size (45 to 57.5 g), with orange peel with a lot of carmine red.

The flesh is orange, with average firmness, intermediate texture, strongly scented, very juicy.

The kernel is of average size, round shaped, adherent to the pulp, sweet core.

The results of the sensory analysis for processed products indicate weaker results for this cultivar, because only in the case of comfiture (18.16 points) tasters have given the"very good" qualificative (Table 6).

Table 6. Sensory analysis of the apricot processed products from the 'Augustin' cultivar

MU=points

Specification	Product			
	Compote	Comfiture	Jam	Nectar
Aspect	5.04	5.76	4.62	4.56
Color	3.52	3.20	3.52	3.04
Taste	5.76	5.52	2.67	5.28
Consistency	3.52	3.68	3.85	3.68
Overall average score	17.84	18.16	14.66	16.56
Qualificative	good	very good	satisfactory	good

The compote and the nectar have received the ''good'' while the jam (14.66 points) has received the ''satisfactory'' qualificative.

Picture 6 presents the four types of canned apricot from the 'Augustin' cultivar.

Figure 6. Apricot processed products from the 'Augustin' cultivar

The **'Litoral'** cultivar's fruit is big, ovoid, yellow-lime with red dots and streaks on the sunny side.

The flesh is yellow, moderately consistent, has a lot of dry substance and pleasant taste.

The products obtained by processing apricots of this cultivar (Table 7 and Figure 7) were well appreciated by tasters who, by the score given, made it possible that comfiture (18.24 points) and jam (18.06 points) receive the''very good'' qualificative and the compote (16.80 points) and nectar (17.04 points), the rating ''good''.

The data shows that the compote as well the nectar has a pleasant aspect and a taste good, but the consistency has dropped below the overall average score.

Depending on the results obtained in processing the form of canned apricots, there were established the destinations of valorization in processed form of the cultivars studied (Table 8).

Table 7. Sensory analysis of the apricot processed products from the 'Litoral' cultivar

MU=points

Specification	Product			
	Compote	Comfiture	Jam	Nectar
Aspect	5.52	5.76	5.32	5.04
Color	3.04	3.04	5.46	3.36
Taste	5.04	5.76	3.38	5.28
Consistency	3.20	3.68	3.90	3.36
Overall average score	16.80	18.24	18.06	17.04
Qualificative	good	very good	very good	Good

Figure 7. Apricot processed products from 'Litoral' cultivar

It finds that cultivar 'Olimp' is very well suitable to the processing into all 4 types of canned analyzed: comfiture, jam, compote and nectar, the resulting product having remarkable sensorial qualities.

Apricots in variety 'Tudor' are less suitable for processing, being able to consume preferably as fresh fruits or possibly as comfiture or nectar.

Table 8. – The destinations of valorization in processed form of apricot varieties

Cultivar	Option		
	I	II	III
'Tudor'	comfiture	nectar	compote, jam
'Sirena'	nectar	comfiture, jam	compote
'Orizont'	compote, nectar	comfiture, jam	
'Olimp'	compote, comfiture, jam, nectar	-	-
'Neptun'	compote, jam, nectar	comfiture	-
'Augustin'	comfiture	compote, nectar	jam
'Litoral'	comfiture, jam	compote, nectar	-

It finds that cultivar ‘Olimp’ is very well suitable to all the processing into four types of canned analyzed: comfiture, jam, compote and nectar, the resulting product having remarkable sensory qualities. The apricots from the ‘Tudor’ cultivar are less suitable for processing, being able to be consumed fresh fruits or, possibly, as comfiture or nectar.

CONCLUSIONS

The apricots from the seven cultivars studied are different in size, shape, color and fresh storage capacity. Although, all these cultivars have in common a pleasant, sweet flavor, which is why they are highly appreciated for fresh consumption. These characteristics also affect the quality of the processed products.
The following cultivars are recommended:

- for obtaining the canned compote ‘Orizont’, ‘Olimp’, ‘Neptun’;
- for the comfiture, the cultivars: ‘Tudor’, ‘Olimp’, ‘Augustin’, ‘Litoral’;
- for the jam, the cultivars: ‘Orizont’, ‘Neptun’, ‘Litoral’;
- for the nectar: ‘Sirena’, ‘Orizont’, ‘Olimp’, ‘Neptun’.

Cultivar ‘Olimp’ is very well suitable to the processing into all 4 types of canned analyzed: comfiture, jam, compote and nectar, the resulting product having remarkable sensorial qualities. The apricots from the cultivar ‘Tudor’ are less suitable for processing, being able to be consumed preferably as fresh fruits or possibly as comfiture or nectar.

REFERENCES

Caplan I., Lamureanu Gh., Alexe Constanta, 2015. Comparative analysis of the quality of fruit, both fresh and processed as compote, of certain cherry tree cultivars. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology, 72(2): 275-276.

Caplan I., Lamureanu Gh., Alexe Constanta, 2016. Suitability for processing of some sweet cherry varieties. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology, 73(1): 7-13.

Lamureanu Gh., Alexe Constanta, Popescu Simona, 2012. Quality evaluation of some clingstone cultivars processed into stewed fruit. Scientific papers. Series B. Horticulture. Vol. LVI, 2012, Bucharest, 127-132.

Lamureanu Gh., Alexe Constanta, Caplan I., 2015. Quality evaluation of processed products of some peach varieties. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology, 72(2): 162-168.

Sarbu S., Beceanu D., Corneanu G., Petre L., Anghel Roxana, Iurea Elena, 2010. Quality evaluation of some sweet cherry cultivars processed into stewed fruit. Lucrari stiintifice, Vol.53, 2010.University of Agricultural Sciences and Veterinary Medicine Iasi – Horticulture Series. 479-484.

Veringă Daniela, Dumitrescu L., 2016. Comparative Methods in determining the quality of peach processed products. Journal of Horticulture, Forestry and Biotechnology, vol 20 (3) Banat University of Agricultural Sciences and Veterinary Medicine Timosoara, 137-141.

Vintila M., Lămureanu Gh., Alexe Constanta, 2015. Aspects regarding the quality of processed products of some cherry varieties – 43nd Symposium "Actual Tasks on Agricultural Engineering", Opatija, Croația, ISSN 1848-4425, Thomson Reuters: Conference Proceedings, 775-785.

STAS 12656-88. Produse alimentare. Analiza senzoriala. Metode cu scari de punctaj. Standard de Stat, Editie oficiala.

STAS 3164-90. Compot de fructe. Standard de Stat, Editie oficiala.

STAS 3750-90. Dulceata. Standard de Stat, Editie oficiala.

STAS 3183–90. Gemuri. Standard de Stat, Editie oficiala.

SP 877-96. Nectar de fructe. Standard Profesional, Editie oficiala.

www.pro-sanatate.com/caisele-beneficii-sanatate/ Caisele – beneficii pentru sanatatea ta

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

A REVIEW OF HOW TO OPTIMIZE STORAGE AND SHELF LIFE EXTENDING TECHNOLOGIE OF KIWIFRUIT (*ACTINIDIA* SP.) BY USING 1-METHYLCYCLOPROPENE TO MEASURABLY REDUCE FRUIT WASTE

Ramona COTRUŢ, Anca Amalia UDRIŞTE

Research Center for the Study of Quality Food Products,
59 Mărăşti Blvd., District 1, 011464, Bucharest, Romania

Corresponding author email: ramona_cotrut@yahoo.com

Abstract

Kiwifruit (Actinidia deliciosa) are capable of long term storage only if carefully protected against deterioration prior to and during storage. They are harvested when mature but unripe and must ripen before eating. They are extremely sensitive to ethylene gas, which causes rapid flesh softening during storage, starch depletions to reduced sugars, increased susceptibility to spread of fruit rotting organisms and physiological breakdown. Hardy kiwifruit/Baby kiwi (Actinidia arguta) have smooth, edible skins and are smaller in size than 'Hayward' kiwifruit. Unlike A. deliciosa, baby kiwi fruits are very sensitive to dryness because of their smooth peels that lack hair. This phenotype characteristic is the main reason for the short-storage time and fast loss of postharvest quality. Fruits are not picked vine ripe, as they would be too soft to package and ship, instead they are picked when physiologically mature and firm, and are stored under refrigeration (0˚C, 90–95% RH). Limited information exists regarding the ripening physiology of hardy kiwifruit or the ideal packaging and storage conditions for optimum quality, storage and shelf life. The objectives of this paper is to integrate existing knowledge and findings about applying technologies developed to suppress ethylene content and its effects degrading the kiwifruit post-harvest by applying 1-methylcyclopropene and periodically reviewing its effects and changes in kiwifruit quality, thereby improving storage technologies and extend the shelf life. The paper is an overview of how to optimize storage technologies of kiwifruit, managing fruit ripening by controlling naturally occurring ethylene during storage for optimal market value and more efficient harvest management, while maintaining excellent quality fruit and reduce losses and findings reveal the importance for assessing the marketing performance of the retail wine stores and the limits that generated lack of adoption on a large scale.

Key words*: kiwifruit, 1-methylcyclopropene, shelf life, short-storage.*

INTRODUCTION

The inhibitor of ethylene, 1-methylcyclopropene (1-MCP), is a new technology that is increasingly being used to improve storage potential and maintain quality of fruit and vegetables. 1-MCP is a synthetic cyclic olefin capable of inhibiting ethylene action. 1-MCP is a cycloalkene with the molecular formula C4H6 (Figure 1). It is a volatile gas at standard temperature and pressure with a boiling point of ~12°C. It acts as a competitor of ethylene, blocking its access to the ethylene-binding receptors (Sisler and Serek, 1997). The affinity of 1-MCP for the receptors is approximately 10-times greater than that of ethylene and, therefore, compared with ethylene, thus it is active at much lower concentrations.

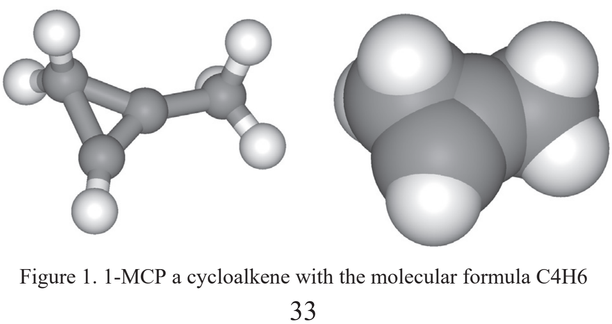


Figure 1. 1-MCP a cycloalkene with the molecular formula C4H6

1-MCP is a gaseous nontoxic product that delays softening and improves post-storage quality of several climacteric fruits (Blankenship and Dole, 2003) and it is applied to extend their postharvest life. To maintain the market position of the fruit, it is very important that techniques of storage and ripening retardant applications be used, which reduce the effect of ethylene (Watkins, 2008) and conserve a longer postharvest quality (Magaña et al., 2004; Andrade et al., 2006). Ethylene is a natural gaseous hormone (Martínez-Romero et al., 2007) that regulates the processes related to fruit ripening and senescence (Binder, 2008). The action of ethylene results from the binding of molecules to receptors located in the cell membrane of the endoplasmic reticulum (Serek et al., 2006). This binding activates the receptors, which send transduction signals, generating a gene expression and physiological response (Pereira et al., 2008). For this reason, the endogenous and/or exogenous presence of this hormone accelerates ripening and creates desirable senescence effects (fast, uniform ripening) or undesirable effects, such as reductions in the fruit's life (Giovannoni, 2004). There is a group of compounds called analogues of ethylene that competes for membrane receptors and inhibits the effect of this hormone, within which 1-methylcyclopropene (1-MCP) is notable, which has been widely used in various fruits and vegetables (Nanthachai et al., 2007; Watkins, 2008), generating changes in multiple metabolic processes, such as decreases in respiratory rates, ethylene production and volatile degradation of chlorophylls, changes of color, sugars, acidity and softening, which vary depending on the fruit, concentration and exposure time (Watkins, 2006). Sooyeon et al. (2015) used the 1-MCP treatment to inhibite hardy kiwifruits (*Actinidia arguta*) ripening by reducing respiration and ethylene production; the hardy kiwifruits could be stored for up to 5 weeks by maintaining higher fruit firmness, ascorbic acid and total phenolic contents, reducing changes in acidity, respiratory rate and color. In this sense, the employment of technologies such as 1-MCP applications has a potential use in the reduction of changes associated with quality losses during postharvest, which would extend the shelf-life of fruits and vegetables (Vilas-Boas and Kader, 2007); so the greater exposure time of the product, the lower the needed concentration will be in order to obtain the desired effect (Bassetto, 2002). Extensive literature about the effects of 1-MCP on fruit, vegetables, and ornamental products exists, and by 2007, results for over 50 fruit and vegetables, both whole and fresh-cut, as well as ornamental products, were available (Watkins and Miller, 2006). Other studies shows that the effect of 1-MCP on fruit considers the effects on factors that influence product quality using several fruit that have received the most attention in the literature, and that highlight some of the challenges that exist in commercialization of 1-MCP-based technology.

THE EFFECTS OF 1-MCP POSTHARVEST TREATMENT

For *Actinidia aruguta* (Seib. et Zucc.) Planch. Ex Miq., known as 'hardy kiwifruit' or 'baby kiwifruit' the recommended storage period of hardy kiwifruits is one to two weeks and an additional two or three days for shelf life. The main reasons for the short storage life are fruit softening, skin wrinkling due to water loss (dryness) and fruit decay. Fruit softening rapidly increases at the room-temperature ripening period, after harvest or cold storage (Krupa et al., 2011). Unlike *A. deliciosa*, the hardy kiwifruits are very sensitive to dryness because of their smooth peels that lack hair. This phenotype characteristic is the main reason for the short-storage time and fast loss of postharvest quality (Strik, 2005).

In various climacteric fruit including kiwifruit, preclimacteric application of 1-methylcyclopropene (1-MCP), a potent inhibitor of ethylene perception due to its largely irreversible binding to ethylene receptors, has been reported to delay ripening and senescence significantly, and consequently to lead to a prolonged storage life and/or shelf life (Watkins, 2006).

To extend the storage life of hardy kiwifruits different edible coating materials were used in past, consisting of mixtures of various formulas, such as calcium caseinate, chitosan, Prima Fresh 50-V and Semperfresh (Fisk et al.

(2008). Krupa et al. (2011) reported that hardy kiwifruits stored in common cold storage gradually lost physicochemical quality over 4 weeks due to decreases in ascorbic. Contrary to the fruit of *A. deliciosa*, which can be stored in cool conditions for up to 5 months, the storage time of hardy kiwifruits is usually no longer than 10–12 weeks and varies from year to year (Strik, 2005).

In Asiche et al. (2016) study, the application of 1-MCP after propylene treatment delayed the initiation and progression of ethylene biosynthesis and overall fruit ripening. 1-MCP extended the "eating window"of kiwifruit, especially in fruit treated with propylene for 48 h that had started ethylene biosynthesis. This suggests that, in kiwifruit, immediately after the commencement of propylene treatment, even before ethylene production is initiated, synthesis of cell-wall degrading enzymes isinitiated, which induces subsequent fruit softening, whereas the application of 1-MCP delayed fruit softening induced by propylene. Similar effects of 1-MCP in delaying fruit softening after ethylene or propylene treatment have also been observed in melon (Nishiyama et al., 2007) and 'La France' pear (Kubo et al., 2003).

In his study Park et. Al., (2015), provides dates where flesh firmness decreased gradually with storage time and reached lower levels at the end stage of storage in 1-MCP treatments. Fruits treated with 1-MCP enhanced firmness, about 20 % higher than control fruits. Application of 1 ppm of 1-MCP was sufficient to delay kiwi fruit softening during cold storage. In Park's experiment it has been shown that flesh firmness enhanced by 1-MCP treatment changes with increasing of starch content during storage. Several explanations are available for the tendency of firmness in correlation with starch content when the treatment was carried out. 1-MCP treated kiwi fruits showed the lesser decrease of the starch content. The relatively high content of starch is related to the enhanced kiwi fruit firmness.

In his study, Kwanhong et. al. (2017), shows that 1-MCP treatment effectively delayed the rate of fruit softening in red-fleshed kiwifruit by suppressing ethylene biosynthesis during storage at all temperatures. The application of 1-MCP was very effective in delaying fruit ripening and senescence, especially at higher storage temperatures above 10°C, according to his study.

As shown in Hwanhong et al. study, 1-MCP treatment also influenced both SSC and TA of red-fleshed kiwifruit. The fruit reached full ripeness when the SSC level increased to 15 Brix. His study demonstrates that application of 1-MCP could delay the increase of SSC and decrease acidity. Fruit treated with 1-MCP had lower SSC and higher TA than untreated fruit during storage at all temperatures, similar to previous studies on 'Hayward'(Koukounaras and Sfakiotakis, 2007) and 'Allison' kiwifruit (Sharma et al., 2012). In which sensory evaluation regards, storage had a measurable effect on the quality of the fruit. The results show that 1-MCP-treated fruit were harder, sourer, and less juicy than untreated fruit, especially for fruit stored at 20°C. This suggests that 1-MCP treatment could delay softening and sourness in fruit stored at all temperatures. However, the 1-MCP treatment did not affect the juiciness of the fruit, except for fruit stored at 20°C which were less juicy.

Boquete et al. (2004) and Kim et al. (2001) determined that application of 1-MCP in kiwi fruits reduces ethylene production and softening during cold storage and subsequently exposed to 20 °C. Low concentrations of 1-MCP from 2.5 ppm to 1 ppm in most commodity are the most effective, but this depends also on temperature treatment. The most commonly applied is 20–25°C, but lower temperatures can be used in some commodities (Mir et al. 2001). Generally, optimal treatment durations of 12–24 h in fruits were sufficient to achieve a full response (Ku and Wills, 1999b). It was shown that 1-MCP effectiveness in fruits and vegetables were influenced by the cultivar, developmental stage, time from harvest to treatment and its concentration (Wills and Ku, 2002).

1- MCP is being used as a powerful tool to gain insights into fundamental processes that are involved in ripening and senescence, as well as to understand ethylene's action and responses (Watkins, 2006). The effects of 1-MCP in fruits are variable depending on the fruit. For example, 1-MCP induced an increase in sugars (expressed as soluble solids) in papaya (Hofman et al., 2001) and pineapple

(Selvarajah et al., 2001), but reduced sugars in kiwifruit (Boquete et al., 2004) and nectarines (Bregoli et al., 2005). Furthermore, 1-MCP had no effect on soluble solid contents of plums (Menniti et al., 2004) and mamey sapote (Ergun et al., 2005). Organic acids such as citric acid were reduced in 1-MCP-treated apple (Defilippi et al., 2004) and were increased in guava (Bassetto et al., 2005); malic acid in apple did not change due to 1-MCP treatment (Defilippi et al., 2004; Kondo et al., 2005).

Respiration rates and ethylene production are reduced in fruits treated with 1-MCP most of the time (Jiang et al., 2001; Dong et al., 2002; Mwaniki et al., 2005). Exposure of kiwifruits to exogenous ethylene (ethephon) accelerates maturation, which generates metabolic processes that reduce postharvest fruit life. The 1-MCP treatment extended the postharvest life of the kiwifruits, slowing the metabolic processes and loss of firmness and likewise decreased the respiration rate. The main method used to prolong the storage life of fruit is through reducing the fruit temperature to slow metabolism. Refrigerated storage slows the rate of ripening and senescence of the fruit, and also slows the development of any rots.

The way in which temperature management is implemented after harvest can significantly affect the quality of the fruit at the end of storage, both in the amount of ripening retardation and also the presence or absence of disorders. The basic effect of refrigerated storage on fruit can be supplemented by modification of the atmosphere in the coolstore, by reducing oxygen and increasing carbon dioxide concentrations. The application of the inhibitor of ethylene action 1-methylcyclopropene (1-MCP) has become common to slow the ripening of a range of fruit. The technologies impact on the fruit is dependent on the physiological state, or maturity, of the fruit at harvest and may differ dependent on the commercial requirements of the fruit, i.e. a short or long storage period.

Ultimately, the target for good storage is for the fruit to remain in good condition, to ripen properly, have an acceptable flavour and not to have any disorders at the end of storage and when it reaches the consumer. For commercial practice it is needed to be taken into consideration the cultivar, the maturity and ripening stage, the time between harvest and treatment, the temperature.

In **summary**, this review supports previous research on the beneficial effects of 1-MCP application in delaying ripening and postharvest quality loss of kiwifruit, and can extend its storage life.

REFERENCES

Andrade J.L., Renfigo E., Ricalde M.F., Simá J.L, Cervera J.C.,Vargas-Soto G., 2006. Microambientes de luz, crecimiento y fotosíntesis de la pitahaya (Hylocereus undatus) en un agrosistema de Yucatán, México. Agrociencia, 40: 687-697.

Asiche W. O, Mworia E. G., Oda C., Mitalo O. W., Owino W. O.,Ushijima K., Nakano R., Kubo Y, 2016. Extension of Shelf-life by Limited Duration of Propylene and 1-MCPTreatments in Three Kiwifruit Cultivars, The Horticulture Journal 85 (1): 76−85.

Bassetto E., 2002. Conservação de goiabas 'Pedro Sato' tratadas com 1-metilciclopropeno: concentrações e tempos de exposição. M.Sc. thesis. Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, Brazil.

Bassetto E., Jacomino A.P., Pinheiro A.L., Kluge R.A., 2005. Delay of ripening of 'Pedro Sato' guava with 1-methylcyclopropene. Postharvest Biol. Technol., 35: 303-308. DOI: 10.1016/j.postharvbio.2004.08.003.

Binder B., 2008. The ethylene receptors: complex perception for a simple gas. Plant Sci., 175: 8-17.

Blankenship S. M., Dole J. M. 2003. 1-Methylcyclopropene: A review. Postharvest Biol. Tech. 28:1–25.

Boquete E.J., Trinchero G.D., Fraschina A.A., Vidella F., Sozzi S.G.O. 2004. Ripening of 'Hayward' kiwi fruit treated with 1- methylcyclopropene after cold storage. Postharv Biol Technol 32: 57–65.

Bregoli A.M., Ziosi V., Biondi S., Rasori A. Ciccioni M., 2005. Postharvest 1-methylcyclopropene application in ripening control of Stark Red Gold nectarines: Temperature dependent effects on ethylene production and biosynthetic gene expression, fruit quality and polyamine levels. Postharvest Biol. Technol., 37: 111-121. DOI: 10.1016/j.postharvbio.2005.04.006.

Defilippi B.G., Dandekar A.M., Kader A.A., 2004. Impact of suppression of ethylene action or biosynthesis on flavor metabolites in apple (*Malus domestica* Borkh) fruits. J. Agric. Food Chem., 52: 5694-5701. DOI: 0.1021/jf049504x

Dong L., Lurie S., Zhou H.W., 2002. Effect of 1-methylcyclopropene on ripening of 'Canino' apricots and 'Royal Zee' plums. Postharvest Biol. 5214(01)00130-2.

Ergun M., Sargent S.T., Fox A.J., Crane J.H., Huber D.J., 2005. Ripening and quality responses of mamey sapote fruit to postharvest wax and 1-methylcyclopropene treatments. Postharvest Biol.

Technol., 36: 127-134. DOI:10.1016/ j.postharvbio.2004.12.002.

Fisk C. L., Silver A., Strik B. and Zhao Y., 2008. Postharvest quality of hardy kiwifruit (*Actinidia aruguta* 'Ananasnaya') associated with packaging and storage conditions. Postharvest Biology and Technology, 47: 338–345.

Giovannoni J.J., 2004. Genetic regulation of fruit development and ripening. Plant Cell, 16: S170-S180.

Hofman P.J., Jobin-Decor M., Meiburg G.F., Macnish A.J., Joyce D.C., 2001. Ripening and quality responses of avocado, custard apple, mango and papaya fruit to 1-methylcyclopropene. Aust. J. Exp. Agric., 41: 567-572. DOI: 10.1071/EA00152.

Jiang Y.M., Joyce D.C., Terry L.A., 2001. 1-Methylcyclopropene treatment affects strawberry fruit decay. Postharvest Biol. Technol., 23: 227-232.DOI: 10.1016/S0925-5214(01)00123-5.

Kim H. O., Hewett E. W., Lallu N. 2001. Softening and ethylene production of kiwi fruit reduced with 1-methylcyclopropene. Acta Horticult 553:167–170.

Kondo S., Setha S., Rudell D.R., Buchanan D.A., Mattheis J.P., 2005. Aroma volatile biosynthesis in apples affected by 1-MCP and methyl jasmonate. Postharvest Biol. Technol., 36: 61-68.

Koukounaras A, Sfakiotakis E., 2007. Effect of 1-MCP prestorage treatment on ethylene and CO2 production and quality of 'Hayward' kiwifruit during shelf-life after short-, medium- and long-term cold storage. Postharvest Biol Technol 46: 174-180.

Krupa T., Latocha P. and Liwin´ ska A., 2011. Changes of physiological quality, phenolics and vitamin C content in hardy kiwifruit (*Actinidia aruguta* and its hybrid) during storage. Scientia Horticulturae, 130: 410–417.

Ku V.V.V., Wills R.B.H., 1999b. Effect of 1-methylcyclopropene on the storage life of broccoli. Postharv Biol Technol 17:127–132.

Kubo Y., K. Hiwasa, Owino W. O., Nakano, Inaba A., 2003. Influence of time and concentration of 1-MCP application on the shelf life of pear 'La France' fruit. HortScience 38: 1414–1416.

Kwanhong P., Lim B. S., Lee J.S., Park H. J, Choi M. H, 2017. Effect of 1-MCP and Temperature on the Quality of Red-fleshed Kiwifruit (Actinidia chinensis), Horticultural Science and Technology 35(2):199-209.

Magaña W., Balbin M., Corrales J., Rodríguez A., Saucedo C., Cañizares E., and Sauri E., 2004. Effects of cold storage on fruit physiological behavior of pitahaya (Hylocereus undatus Haworth). Cultivos Tropicales, 25: 33-39.

Martínez-Romero D., Bailen G., Serrano M., Zapata P., Castillo S., and Valero D., 2007. Tools to maintain postharvest fruit and vegetable quality through the inhibition of ethylene action. A review. Crit. Rev. Food Sci. Nutr., 47:543-560.

Menniti A.M., Gregori R. Donati I., 2004. 1-methylcyclopropene retards postharvest softening of plums. Postharvest Biol. Technol., 31: 269-275. DOI: 10.1016/j.postharvbio.2003.09.009.

Mir N.A., Curell E., Khan N., Whitaker M., Beaudry R. M., 2001. Harvest maturity, storage temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Redchief Delicious' apples. J Amer Soc Horticult Sci 126:618–624.

Mwaniki M.W., Mathooko F.M., Matsuzaki M., Hiwasa K., Tateishi A., 2005. Expression characteristics of seven members of the betagalactosidase gene family in 'La France' pear (Pyrus communis L.) fruit during growth and their regulation by 1-methylcyclopropene during postharvest ripening. Postharvest Biol. Technol., 36: 253-263. DOI:10.1016/j.postharvbio.2005.02.002.

Nanthachai N., Ratanachinakorn B., Kosittrakun M., Beaudry R.M., 2007. Absorption of 1-MCP by fresh produce. Postharv. Biol. Technol., 44:291-297.

Nishiyama I. 2007. Fruits of the Actinidia genus. Adv. Food Nutr. Res. 52: 293−324.

Park Y. S., Myeng H. I., Gorinstein S., 2015. Shelf life extension and antioxidant activity of 'Hayward' kiwi fruit as a result of prestorage conditioning and 1-methylcyclopropene treatment . J Food Sci Technol (May 2015) 52(5):2711−2720.

Pereira, G., Luiz F., Dias V., Brommonschenkel S., 2008. Influéncia do tratamento com etileno sobre o teor de sólidos solúveis e a cor de pimentas. Bragantia, 67:1031-1036.

Selvarajah S., Bauchot A.D., John P., 2001. Internal browning in cold-stored pineapples is suppressed by a postharvest application of 1- methylcyclopropene. Postharvest Biol. Technol., 23: 167-170. DOI: 10.1016/S0925-5214(01)00099-0.

Serek M., Woltering E.J., Sisler E.C., Frello S., Sriskandarajah S., 2006. Controlling ethylene responses in flowers at the receptor level. Biotechnol. Adv., 24:368-381.

Serna, L., Torres L.A, and Ayala A., 2011. Efecto del empaque y del 1-MCP sobre características físicas, químicas y fisiológicas de pitahaya amarilla. Biotecnología en el Sector Agropecuario y Agroindustrial, 9:139-149.

Sharma RR, Jhalegar MJ, Pal RK, 2012. Response of kiwifruit (Actinidia deliciosa cv. Allison) to postharvest treatment with 1-methylcyclopropene. J Hortic Sci Biotechnol 87: 278-284.

Sisler E.C., Serek M., 1997. Inhibitors of ethylene responses in plants at the receptor level: Recent developments, Physiologia Plantarum, 100, pp. 577–582.

Sooyeon S. H. H., Jeongyun K., Han J. L., Jeong G. L., Eun J. L., 2015, Inhibition of hardy kiwifruit (*Actinidia aruguta*) ripening by 1-methylcyclopropene during cold storage and anticancer properties of the fruit extract, Food Chemistry, Volume 190, 1 January 2016, Pages 150–157.

Strik B., 2005. Growing kiwifruit. A Pacific Northwest Extension Bull. 507. Corvallis, Oregon. State University, Extension Service.

Vilas-Boas E., Kader A., 2007. Effect of 1-methylcyclopropene (1-MCP) on softening of fresh-cut kiwifruit, mango and persimmon slices. Postharv. Biol. Technol., 43:238-244.

Watkins C.B., 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol. Adv., 24:389-409.

Watkins C.B., 2008. Postharvest effects on the quality of horticultural products: Using 1-MCP to understand the effects of ethylene on ripening and senescence processes. Acta Hort., 768:19-31.

Wang K.L.C., Li H., and Ecker J.R., 2002. Ethylene biosynthesis and signalling networks. Plant Cell, 14: S131-S151.

Wills R. B. H, Ku V. V. V., 2002. Use of 1-MCP to extend the time to ripen of green tomatoes and postharvest life of ripe tomatoes. Postharv Biol Technol 26:85–90.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPOSITION OF PHENOLIC COMPOUNDS IN PETAL FLOWERS AND LEAF SAMPLES OF VARIOUS APPLE CULTIVARS

Fatma YILDIRIM[1], Adnan Nurhan YILDIRIM[1], Tuba DILMAÇÜNAL[1], Bekir SAN[1], Nilda ERSOY[2]

[1]Suleyman Demirel University, Agriculture Faculty, Department of Horticulture, 32260, Isparta, Turkey
[2]Akdeniz University, Technical Sciences Vocational School, 07058, Campus/Antalya, Turkey

Corresponding author email: fatmayildirim@sdu.edu.tr

Abstract

The phenolic compounds of petals and leaf tissue samples were determined in apple cvs. 'Breaburn', 'Golden Reinders', 'Granny Smith' and 'Jonagold ' grafted on M9; 'Summerred ' and 'William's Pride' grafted on M.26. Petals of apple flowers were taken at the pink bloom floral stage in April. Moreover leaves were sampled from the middle part of the annual shoots in July. The phenolic compounds were analyzed by High Pressure Liquid Chromatography (HPLC) technique. The gallic acid, p-hydroxy benzoic acid, eriodictyol, quercetin, ferulic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid and apigenin-7-glucoside contents were determined in petal samples. The gallic acid, p-hydroxy benzoic acid, eriodictyol, quercetin, ferulic acid and p-coumaric acid were also investigated in leaf tissues. The concentrations of the compounds were influenced by the genotypes as well as by tissue samples. While eriodictyol was the predominant phenolic compound in leaves ranging between 156.75-414.90 µg/g DW, chlorogenic acid was the predominant phenolic compound found only in petals ranging between 7784.60-19293.00 µg/g DW in all cultivars investigated in this research. It was determined that the petals of apples were quite richer than the leaf samples in terms of the phenolic contents. Among the studied cultivars the total concentrations of phenols were higher in both the petal and leaf tissues of 'Granny Smith' apple cultivar.

***Key words**: Malus, cultivar, leaves, petal, phenolic.*

INTRODUCTION

Phenolics are aromatic compounds in which one (phenol) or more (polyphenol) hydroxyl groups are bound to a benzene ring and constitute a significant part of the biochemical components of the plant. They represent the most abundant widely spread class of plant biochemicals. They are involved in a number of physiological functions in plants. For example, flavonoids which are a large and diverse group of phenolic compounds, act as attractants for pollination and seed dispersal (Downey et al., 2006) and flavonols also help to facilitate conditional male fertility in pollen by providing pollen tube growth (Pollak et al., 1995). On the other hand phenolic compounds can protect the plant against UV-light, insects and pathogens (Vermerris and Nicholson, 2008) and have a role in mechanical support by lignification and affect the growth of neighboring plants (allelopathy) (Özeker, 1999).

Plant phenolics have positive effects on human health and nutrition (Vermerris and Nicholson. 2008), and are the basis of several plant-derived drugs and cosmetic products. Many researchers have also reported that plants are rich sources of polyphenols which have bioactive effect in human such as antioxidant (Pandey and Rizvi, 2009), antimicrobial activities (Rauha et al., 2000), anti-glycemic (Rizvi and Zaid, 2001) and anti-cancer (Yang et al., 2001), anti-inflammatory (Zhu et al., 2015) etc. Therefore, studies on the definition, extraction and purification of these compounds from different plant organs have been increasing. In this regard determination of the composition and content of phenolic compounds in different plant vegetative organs are important.

Apple (*Malus x domestica* Borkh.) is one of the most produced fruit species in the world. Its fruits are known as rich sources of phenolics, especially catechins, procyanidines, phloretin glycosides and chlorogenic acid (Matthes and Schmitz-Eiberger, 2009). Apple leaves are also rich in terms of phloridzin in which exhibit antidiabetic activity and quercetin glycosides (Liaudanskas et al., 2014). Although differences in phenolic contents of cultivars have been shown in apple leaves and fruits, there is no research on phenolic compounds of apple flowers. It is known that apple flower tea is recommended for facilitating digestion and for enhancing skin.

The aim of this study was to compare the contents of phenolic components in different apple cultivar petals and leaves to evaluate their potential as sources of bioactive compounds as well as genetic differences.

MATERIALS AND METHODS

The biological material. The trials were carried out at the experimental apple (*Malus x domestica* Borkh.) orchard of Suleyman Demirel University, Agricultural Research and Application Center located in Isparta, Turkey (37º50'23"N 30º32'02"E) in 2007. All of the trees used as plant material were planted in 2003 with 1x3 m spacing and trained as modified central leader system. The petals of the flower samples were taken at the stage of pink bloom early in the morning in April. Leaf samples were taken from the middle of one year old shoots all around the tree in July. The collected samples were brought to the laboratory immediately, washed under tap water, rinsed with distilled water, put into paper bags, and dried in an air-blowing drying oven set at 65°C. The dried samples were ground to powder with a blender. Trial I: Determining the phenolic compounds of petals and leaves of "Breaburn", "Golden Reinders", "Granny Smith" and "Jonagold" apple cultivars grafted on M9 rootstock. Trial II: Determining the phenolic compounds of petals and leaves of "Summerred" and "William's Pride" apple cultivars grafted on M26 rootstock.

Determination of phenolic compounds. Phenolic compounds were analyzed by the modified procedure of Escarpa and González (1998). 25 ml of acetone-water solution (80 % acetone and 20 % water v/v) was added to 2.5 g of ground samples. The upper phase was taken in a centrifuge tube after the extract was incubated in a water bath at 50°C for 30 min. Then, the extraction was repeated twice using 25 ml of acetone-water solution each time and the extract was incubated in a water bath at 50°C for 30 min and the upper phase was added to the centrifuge tube again. These combined phases were centrifuged at 10.000 rpm for 5 min. The solvent was evaporated at 40°C under vacuum and samples were re-dissolved in 2 ml of methanol. Solutions were filtered by membrane filters with a pore size of 0.45 µm and then 20 µl of the solutions was injected into HPLC (High Pressure Liquid Chromatography). HPLC analysis was performed using a Shimadzu HPLC system with a diode array detector (DAD λmax=278). The column used was an Agilent Eclipse XDB-C_{18} (250x4.60 mm 5 µm) operated at 30°C. Mobile phase: Solvent A (2 % solution of acetic acid in water)—Solvent B (Methanol). Flow rate: 0.8 ml min^{-1}, Injection volume: 10 µl. Peak identification was done according to the standards (*p*-hydroxybenzoik acid, eriodictyol, ferulic acid, *p*-coumaric acid, gallic acid, quercetin, apigenin 7-glucoside, chlorogenic acid, syringic acid, caffeic acid, rosmarinic acid, epicatechin, catechin, rutin, resveratrol, hesperidin, naringenin, luteolin, apigenin and acacetin). The phenolic standards were purchased from Sigma Chemical Co. The concentration of phenolics was expressed as µg g^{-1} dry matter.

Statistical analysis. The data were subjected to the analysis of variance (ANOVA) by using the Minitab software program, and the means were separated by Duncan's Multiple Range Test (5%).

RESULTS AND DISCUSSIONS

Phenolic compounds of petals. The obtained results are presented in Table 1 and Table 2. These data indicated that the petals had the higher phenolic compounds than the leaves of all apple cultivars investigated in this research. Zou et al. (2011) also reported that the petals of loquat were rich in terms of total phenolics and total flavonoids. In our study, a total of 10 kinds of phenolic compounds found in petals of apple flower samples were identified and quantified. The highest amount of phenolic compound determined in petals was the chlorogenic acid followed by apigenin-7-glucoside, quercetin, caffeic acid, eriodictyol, *p*-coumaric acid, syringic acid, gallic acid, *p*-hydroxybenzoic acid and ferulic acid.

Table 1. Average concentration of phenolic compounds in petal samples of four apple cultivars grafted on M9 in Trail 1 (μg/g DW).

Phenolic compound	Cultivars				
	Breaburn	Golden Reinders	Granny.Smith	Jonagold	Average
Phenolic acids					
clorogenic acid	12208.00 b*	9840.10 c	19293.00 a	12877.00 b	13554.53
caffeic acid	160.65 d	203.25 c	400.50 a	349.30 b	278.43
p-coumaric acid	28.26 d	99.77 b	146.60 a	95.49 c	92.53
syringic acid	40.19 c	100.02 b	111.75 ab	116.45a	92.10
gallic acid	89.70 b	108.40 a	83.07 c	63.98 d	86.29
p-hydroxybenzoik acid	71.50 b	29.11 c	60.34 b	141.90 a	75.71
ferulic acid	25.27 b	nd	48.54 a	nd	36.91
Flavonoids					
apigenin-7-glucoside	1272.60 c	1728.10 b	1383.90 c	2222.40 a	1651.75
quercetin	307.50 b	894.20 a	303.35 bc	236.50 c	435.39
eriodictyol	116.65 b	103.15 bc	457.25 a	95.58 c	193.16
Total	14320.32	13106.10	22288.30	16198.60	16496.78

*Means with different superscripts in the same line are statistically significantly different ($p<0.05$), nd: not detected.

Table 2. Average concentration of phenolic compounds in petal samples of two apple cultivars grafted on M26 in Trail 2 (μg/g DW).

Phenolic compound	Cultivars		
	Summerred	William's Pride	Average
Phenolic acids			
clorogenic acid (phenolic acid)	7784.60 b*	7997.60 a	7891.10
caffeic acid	191.50 b	197.15 a	194.325
p-coumaric acid	130.25 a	44.07 b	87.16
syringic acid	81.00 a	61.54 b	71.27
gallic acid	90.91 b	94.11 a	92.51
p-hydroxybenzoik acid	110.30 a	77.60 b	93.95
ferulic acid	276.95 b	343.30 a	310.125
Flavonoids			
apigenin-7-glucoside	1735.80 a	1132.80 b	1434.30
quercetin	214.25 a	137.50 b	175.88
eriodictyol	16.99 b	23.17 a	20.08
Total	10632.55	10108.84	10370.70

*Means with different superscripts in the same line are statistically significantly different ($p<0.05$), nd: not detected.

These results revealed that the petals of apple flowers have a strong antioxidant capacity. Likewise a positive relationship was detected between the phenolics and antioxidant capacities of loquat flowers (Liaudanskas et al., 2014). To our knowledge there is no literature on the phenolic compounds of petals of apple flowers. Therefore, this research is also important in terms of being the first in its field. The results indicated that the contents of each phenolic component of petals were affected by the cultivars. Similarly Zhou et al. (2011) found differences between the contents of flavonoids and phenolics of the flowers of five loquat cultivars. "Granny Smith" had the highest values for total amount of the phenolic components detected in the petals, whereas the lowest value was found in "Golden Reinders" in trail 1 (Table 1). "Granny Smith" is a self-fertile, good pollinator and high productive apple cultivar. The high phenolic contents of this cultivar can be the reason for its high fertility. Likewise, it is reported that attraction may occur through secondary phenolic compounds (flavonoids) in the petals (Shirley, 1996; Özeker, 1999). Thus phenolics may increase the fruit set and yield by playing a role in pollination as well as pollen tube growth. The contents of clorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid and eriodictyol were the highest in "Granny Smith" (Table 1). While the contents of gallic acid and quarcetin were the highest in "Golden Reinders", the contents of *p*-hydroxybenzoik acid, syringic acid and apigenin-7-glucoside were the highest in "Jonagold". In trial 2, total amount of the phenolic components of "Summerred" and "William's Pride" cultivars were found close to each other and the lowest than the cultivars investigated in trail 1. The contents of ferulic acid of both cultivars were quite higher than the other cultivars evaluated in trail 1, while the contents of eriodictyol and quercetin were quite lower. The results of this research emphasized that ferulic acid, eriodictyol and quercetin found in the petal are affected by the rootstocks as well as cultivars.

Phenolic compounds of leaves. The obtained results are presented in Table 3 and Table 4. The phenolic contents of the leaf samples were found lower than the petals of the apple cultivars. Totally 6 kinds of phenolic compounds were evaluated in this research. The highest amount of phenolic compound obtained in leaf samples was eriodictyol followed by ferulic acid, quercetin, *p*-hydroxybenzoic acid, gallic acid and *p*-coumaric acid. Similarly gallic acid, ferulic acid and *p*-coumaric acid were found in leaf samples of apple by Tao et al. (2008) and Petkovsek et al. (2009). Eriodictyol, the highest amount of phenolic compound obtained in this research from the leaf samples of apples, is known as a flavanone and has long been considered as an antioxidant and anti-inflammatory agent. Thus, apple leaves may be considered as a potential antioxidant for human diseases such as acute lung injury (Zhu et al., 2015). The results of this research indicated that the content of each phenolic component obtained from the leaf samples of apples were affected by cultivar. Likewise, many researchers reported that the phenolic contents of the apple vary by cultivar (García et al., 2004; Mikulič-Petkovšek et al., 2004; Usenik et al., 2004; Petkovsek et al., 2009). According to the total results, the highest amount of phenolic components were found in "Granny Smith" followed by "Golden Reinders", "Jonagold" and "Breaburn" in trail 1 (Table 3), while "William's Pride" had the highest amount of these components in trail 2. The highest amount of *p*-hydroxybenzoic acid was found in "Granny Smith" and *p*-coumaric acid was found only in this cultivar. The highest amount of eriodictyol, quercetin, and ferulic acid were found in "Golden Reinders", while the amount of gallic acid was the highest in "Jonagold". The highest amount of *p*-hydroxybenzoik acid, quercetin and ferulic acid were found in "William's Pride", while the amount of gallic acid was the highest in "Summerred" in trail 2.

Table 3. Average concentration of phenolic compounds in leaf samples of four apple cultivars grafted on M9 in Trail 1 (μg/g DW).

Phenolic compound	Cultivars				
	Breaburn	Golden Reinders	Granny Smith	Jonagold	Average
Phenolic acids					
p-coumaric acid	nd	nd	17.58	nd	17.58
gallic acid	56.82 b*	47.19 bc	38.72 c	71.38 a	53.53
p-hydroxybenzoik acid	35.88 c	nd	131.20 a	48.20 b	89.70
ferulic acid	63.08 c	96.13 a	90.05 a	87.40 ab	84.17
Flavonoids					
quercetin	60.46 c	77.97 a	70.67 ab	63.42 b	68.13
eriodictyol	197.90 b	414.90 a	338.90 a	156.75 b	277.11
Total	378.26	636.19	687.12	427.15	590.22

*Means with different superscripts in the same line are statistically significantly different ($p<0.05$), nd: not detected.

Table 4. Average concentration of phenolic compounds in leaf samples of two apple cultivars grafted on M26 in Trail 2 (μg/g DW).

Phenolic compound	Cultivars		
	Summerred	William's Pride	Average
Phenolic acids			
gallic acid	49.92 a*	39.33 b	44.63
p-hydroxybenzoik acid	34.26 b	43.85 a	39.06
ferulic acid	53.71 b	91.18 a	72.45
Flavonoids			
quercetin	65.51 b	97.63 a	81.57
Eriodictyol	239.90	238.25	239.08
Total	443.30	510.24	572.12

*Means with different superscripts in the same line are statistically significantly different ($p<0.05$).

CONCLUSIONS

As a result, according to the results obtained from this research, the phenolic compounds of the apple vary by the cultivar and the parts of the plant. It was determined that the petals of an apple cultivar were quite richer than the leaf samples in terms of the phenolic contents. While chlorogenic acid was the predominant phenolic compound and found only in petals, eriodictyol was the predominant phenolic compound of leaf samples of apple. Among the studied cultivars, the total concentration of phenols was found higher in both of the tissues of "Granny Smith" apple.

REFERENCES

Downey M.O., Dokoozlian N.K., Krstic M.P., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A Review of Recent Research. Am. J. Enol. Vitic. 57 (3): 257-268.

Escarpa A., Gonzalez M.C., 1998. High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. Journal of Chromatography A. 823: 331-337.

Garcia E., Rom C.R., Murphy J.B., 2004. Comparison of phenolic content of 'Liberty' apple (*Malus* × *domestica*) on various rootstocks. Acta Horticulturae 658: 57-60.

Liaudanskas M., Viskelis P., Raudonis R., Kviklys D., Uselis N., Janulis W., 2014. Phenolic composition and antioxidant activity of *Malus domestica* leaves. The Scientific World Journal. Article ID 306217: 10.

Matthes A., Schmitz-Eiberger M., 2009. Polyphenol content and antioxidant capacity of apple fruit: Effect of cultivar and storage conditions. Journal of Applied Botany and Food Quality 82: 152 – 157.

Özeker E., 1999. Phenolic compounds and their importance. Anadolu. J. of AARI 9 (2): 114-124.

Pandey K. B., Rizvi S.I., 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity 2 (5): 270-278.

Petkovsek M.M., Stampar F., Veberic R., 2009. Seasonal changes in phenolic compounds in the leaves of scab-resistant and susceptible apple cultivars. Canadian Journal of Plant Science 89 (4):745-753.

Pollak R.E., Hansen K., Astwood J.D., Taylor L.R., 1995. Conditional male fertility in maize. Sex Plant Reprod 8: 231-241.

Rauha J.P., Remes S., Heinonen M., Hopia A., Kahkonen M., Kujala T., Pihlaja K., Vuorela H., Vuorela P., 2000. Antimicrobial effects of Finnish plant extracts containing flavonoidsand other phenolic compounds. International Journal of Food Microbiology 56: 3–12.

Rizvi S.I., Zaid M.A., 2001. Intracellular reduced glutathionecontent in normal and type 2 diabetic erythrocytes: Effect of insulin and (-) epicatechin. J Physiol Pharmacol. 52: 483-8.

Shirley B.W., 1996. Flavonoid biosynthesis: "new" functions for an "old" pathway. Trends Plant Science 1: 377-382.

Tao F., Xue S.C., Yan M.Z., Chun Y.Z., Xiao Y.Z., Chuan J.W., 2008. Antioxidation and phenolic constituents in Xinjiang wild apple [*Malus sieversii* (Lebed.) Roem.] leaf. Scientia Agricultura Sinica 41(8):2386-2391.

Usenik V., Mikulic-Petkovsek M., Solar A., Stampar F., 2004. Flavonols of leaves in relation to apple scab resistance. Zeitschriftfür Pflanzen Kkrankheiten und Pflanzenschutz 111(2):137-144.

Vermerris W., Nicholson R., 2008. Phenolic compound biochemistry. Springer Science+Business Media B.V. e-ISBN: 978-1-4020-5164-7.

Yang C.S., Landau J.M., Huang M.T., Newmark H.L., 2001. Inhibition of carcinogenesis by dietary polyphenolic compounds. Ann Rev Nutr. 21:381-406.

Zhou C., Sun C., Chen K., Li X., 2011. Flavonoids, phenolics and antioxidant capacity in the flower of *Eriobotrya japonica* Lindl.Int. J. Mol. Sci. 12: 2935-2945.

Zhu G.F., Guo H.J., Huang Y., Wu C.T., Zhang Z.F., 2015. Eriodictyol a plant flavonoid attenuates LPS induced acute lung injury through its antioxidative and antiinflammatory activity. Experimental and Therapeutic Medicine 10: 2259-2266.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE DETERMINATION OF POMOLOGICAL AND TOTAL OIL PROPERTIES OF SOME OLIVE CULTIVARS GROWN IN ISPARTA, TURKEY

Adnan Nurhan YILDIRIM[1], Fatma YILDIRIM[1], Gülcan ÖZKAN[2], Bekir ŞAN[1], Mehmet POLAT[1], Hatice AŞIK[2], Tuba DILMAÇÜNAL[1]

[1]Suleyman Demirel University, Faculty of Agriculture, Horticultural Science, 32260, Isparta, Turkey
[2] Suleyman Demirel University, Faculty of Engineering, Food Engineering Department, 32260, Isparta, Turkey

Corresponding author email: adnanyildirim@sdu.edu.tr

Abstract

The aim of this study was to determine the physical characteristics of three olive cultivars' fruits at 3 different harvest time (skin green with pink spots, pulp white-skin black, skin black and pulp purple) growing in Mediterrenean region of Turkey in Isparta/Sütçüler at the same garden and growing conditions. Ayvalık and Memecik olive cultivars are grown in large areas of Turkey. The third cultivar Topakaşı, is a local cultivar and is cultivated limitedly in the research area. Thus, in the study, the differences between the varieties adapted to the region's ecology and the varieties brought from different regions were investigated. According to mean values, the highest individual fruit weight was found in Memecik (4.99 g) followed by Topakaşı (3.49 g) and Ayvalık (3.48 g). Ayvalık had the lowest (0.65 g) kernel weight followed by Memecik (0.76 g) and Topakaşı (0.86 g). In terms of fruit / kernel ratio, the Memecik cultivar has the best result (84.77%). The highest amount of dry matter was found in Topakaşı (53.37 g/100 g), followed by Ayvalık (39.00 g/100 g) and Memecik (38.53 g/100 g). The total amount of oil was highest in Ayvalık (57.46 g/100 g), followed by the Memecik (54.19 g/100 g) and Topakaşı (53.84 %).

Key words: *olive cultivars, physical characteristics, Ayvalık, Memecik, Topakaşı.*

INTRODUCTION

The olive (*Olea europaea*) is a native to the coastal areas of the eastern Mediterranean Basin and it is estimated that the cultivation of olive trees began more than 7000 years ago (Ercişli et al., 2012). Olive production in the Mediterranean basin accounts for more than 95 % of world's olive production. Located on the northeastern coast of the Mediterranean Sea, Turkey is a major olive-producing country. Olives originated from the coast of Eastern Mediterranean Sea and, to date, more than 1250 cultivars have been recognized worldwide for olive production. Most of these cultivars are present in countries located in the Mediterranean basin. The presence of 87 local olive cultivars has been documented in Turkey (İpek et al., 2012).
The increasing health consciousness and more cosmopolitan society explains the rising consumption of olive oil around the world and hence the rapid growth of the olive industry. The beneficial health properties of olive oil have been known for centuries, particularly in the Mediterranean region. Olives and olive oil are an inherent part of Mediterranean culture and diet, and hence the decreased incidence of cardiovascular disease in this area (being one of the lowest in the Western Hemisphere) has been attributed to their consumption (Ryan and Robards, 1998). The positive effects of olive oil on health are linked to the presence of monounsaturated fatty acids (oleic acid) and a high antioxidant source, as well as high vitamin (A, D, E, K) content (Oktar et. al., 1983; Ryan and Robards, 1998; Salvador et. al., 2003). It also contains leucine, aspartic acid and glutamic acid, among other essential amino acids. Olive oil is the only vegetable oil that can be consumed without being refined (raw) and has its own odor, color and texture. These properties of olive oil are determined by chemical constituents such as fatty acids, phenolic substances, tocopherols, carotenoids and chlorophyll (Servili and Montedoro, 2002;

Ayton et. al., 2006; Turaa et. al., 2007). The chemical composition of olive oil is highly influenced by genotype, geographical region and its ecological conditions, cultural processes, harvest time and oil extraction methods (Mousa et. al., 1996; Boskou, 2000; Ayton et. al., 2006; Selvili et al., 2007; Al-Maaitah, 2009; Keçeli, 2013). In this study, it was aimed to determine the physical characteristics and oil yield of three olive cultivars at 3 different harvest periods (Pink spots on green ground, pink-violet, purple-black) grown in the same orchard and maintenance conditions in Sütçüler/Isparta located in Mediterranean Region of Turkey. The two of the olive varieties investigated in this research (Ayvalık and Memecik) are the most grown varieties and are grown in large geographical areas in Turkey. The third (Topakaşı) is a local cultivar and grown only in the research area. Thus, in the study, the differences between the cultivars adapted to the region ecology and brought from different regions are also revealed.

MATERIALS AND METHODS

The study was carried out in Sütçüler / Isparta (37°29'40"N 30°58'54"E) located in Mediterranean Region of Turkey. Ayvalık, Memecik and Topakaşı, grown in the commercial orchard conditions where the same cultural practices were applied (irrigation, fertilization, pruning, etc.), were used as plant material. The trees are 10 year old and the planting spacing is 5x4 m. The altitude of the orchard is 250 m. Fruit samples were taken at 3 different stages of maturity according to the coloring of fruit peel and fruit flesh. These are; (1) Pink spots on green ground (Maturity index: 2-3), (2) pink-violet (maturity index: 4-5), and (3) purple-black (maturity index: 6-7). The fruits were harvested by hand. Samples were brought to the laboratory immediately after harvest on ice. Fruit weight (g), fruit width (mm), fruit length (mm), shape index, seed weight (g), seed length (mm) , seed width (mm) and seed/fruit flesh ratio (%) were measured. Dry matter and total oil ratios were determined in fruit samples as well. The measurements were made at each harvest period with 50 fruit samples in each triplicates.

Dry matter. The flesh of olive fruit samples were dried at 105°C in a vacuum oven until the weight reached to a constant weight. The amount of dry matter was calculated as %.

Total oil. 2 g of dried and milled fruit flesh sample was extracted with 200 ml of hexane for 4 hours in a Soxhlet apparatus and then evaporated (Guinda et al., 2003). The total crude oil was calculated as % dry sample.

RESULTS AND DISCUSSIONS

Cultivar and harvest period interactions were found statistically significant in terms of fruit weight (Table 1). As the maturity progressed, statistically significant increase was found in the fruit weight of Memecik. The highest fruit weight was determined in the second period of fruit harvest in Ayvalık.

Table 1. Some fruit characteristics of cultivars

Cultivars	Harvest Period I	Harvest Period II	Harvest Period III	**Mean**
	Fruit Weight (g)			
Ayvalık	3.49bAB	3.66bA	3.30cB	3.48
Memecik	4.69aB	5.09aA	5.19aA	4.99
Topakaşı	3.40b	3.39b	3.67b	3.49
Mean	3.86	4.05	4.05	**Lsd:0.3202**
	Fruit Height (mm)			
Ayvalık	19.17b	19.47b	18.57c	19.07
Memecik	25.45aB	27.19aA	26.59aAB	26.41
Topakaşı	19.94b	19.85b	20.33b	20.04
Mean	21.52	22.17	21.83	**Lsd:1.513**
	Fruit Width (mm)			
Ayvalık	14.78b	14.89b	14.59b	14.76
Memecik	16.20aB	17.45aA	17.55aA	17.07
Topakaşı	14.11b	13.89b	14.40b	14.13
Mean	15.03	15.41	15.52	**Lsd:1.125**

Each value is expressed as mean ±standard deviation. Means followed by different capital letters (years) in the row are significantly different ($p<0.05$). Means followed by different small letters in the columns (cultivars) are significantly different ($p<0.05$).

The highest fruit weight for the Topakaşı was determined in the third period. However, the differences between the harvest periods were not significant. Memecik had the biggest fruits (4.99 g) in all of the three harvest periods. The fruit sizes of Ayvalık and Topakaşı were similar. There was a significant difference in the fruit length and fruit width between the harvest periods only for Memecik. While the highest fruit length (27.19 mm) was detected in the second harvest period, the highest fruit width was found in the third harvest period (17.55 mm).

A significant difference was found between the cultivars in terms of seed weight. The highest average seed weight (0.86 g) was determined in the Topakaşı.

Table 2. Some seed characteristics of cultivars

Cultivars	Harvest Period I	Harvest Period II	Harvest Period III	**Mean**
	Seed Weight (g)			
Ayvalık	0.73	0.61	0.61	0.65b
Memecik	0.73	0.78	0.76	0.76ab
Topakaşı	0.89	0.84	0.86	0.86a
Mean	0.78	0.74	0.74	**Lsd:0.1663**
	Seed Height (mm)			
Ayvalık	13.03c	12.33c	12.17c	12.51
Memecik	18.00aB	18.00aB	19.87aA	18.62
Topakaşı	14.80bB	14.13bB	17.07bA	15.33
Mean	15.28	14.82	16.37	**Lsd:1.605**
	Seed Width (mm)			
Ayvalık	6.56ab	6.33ab	6.33b	**6.41**
Memecik	5.86bB	6.00bB	8.27aA	**6.71**
Topakaşı	6.83aB	6.83aB	8.73aA	**7.47**
Mean	**6.42**	**6.39**	**7.78**	**Lsd:0.7267**
	Fruit flesh/seed ratio (%)			
Ayvalık	79.08	83.33	81.52	81.32
Memecik	84.43	84.68	85.36	84.77
Topakaşı	73.82	75.22	76.57	75.36
Mean	**79.11**	**81.08**	**81.15**	

Each value is expressed as mean ±standard deviation. Means followed by different capital letters (years) in the row are significantly different ($p<0.05$). Means followed by different small letters in the columns (cultivars) are significantly different ($p<0.05$).

There was no significant relationship between seed weight and harvesting periods. While there was a significant increase in the third harvest period in the seed length and width parameters of Memecik and Topakaşı, a insignificant decrease was determined in Ayvalık..

While the longest seed size was determined in Memecik (18.62 mm), the largest seed width (7.47 mm) was found in Topakaşı. As the harvest progressed, the fruit flesh ratio increased.

The highest fruit flesh ratio was found in Memecik (84.77%) and lowest was in Topakaşı (75.36%) (Table 2). Significant differences were found between the cultivars in terms of leaf characteristics. The highest leaf area was determined in Topakaşı (Table 3).

Table 3. Leaf characteristics of cultivars

Cultivars	Leaf Length (mm)	Leaf Width (mm)	Leaf Area (mm^2)
Ayvalık	55.72b	15.02	470.80b
Memecik	55.21b	14.57	619.60ab
Topakaşı	66.50a	16.85	841.90a
Mean	59.14	15.48	644.10
LSD	6.083	2.207	313.5

The differences among the averages indicated with different letters in each column are statistically significant at the level of 5 %

Cultivar and harvest period interactions were found significant in terms of dry matter and total oil (Table 4).

The results of the study showed that the dry matter accumulation varies at different harvest periods according to the cultivars.

The amount of dry matter increased with increasing maturity in Memecik and the highest value was found in the third harvest period when the fruits were the most mature.

A fluctuation was found in Ayvalık and the highest amount of dry matter was determined at the first and third harvest periods and the values were close to each other.

According to the average values, Topakaşı had the highest (over 50%) dry matter content. The results of the research showed that Topakaşı had the ability to accumulate high dry matter in the early harvest period. The reason for this is

thought to be the result of more photosynthesis due to the higher leaf area of Topakaşı. It is observed that Ayvalık also completed the accumulation of dry matter in the early period.
As expected, the total amount of oil increased in all of the three cultivars as the maturity progressed (Table 4).

Table 4. Some chemical characteristics of cultivars

Cultivars	Harvest Period I	Harvest Period II	Harvest Period III	**Mean**
		Dry Matter (g/100 g)		
Ayvalık	40.93bA	36.89bB	39.20cA	**39.00**
Memecik	34.62cC	37.45bB	43.51bA	**38.53**
Topakaşı	54.85aA	52.56aB	52.72aAB	**53.37**
Mean	**43.47**	**42.30**	**45.14**	**Lsd:2.183**
		Total Oil (g/100 g dry matter)		
Ayvalık	51.10aC	57.92aB	63.35aA	57.46
Memecik	46.50bC	53.80bB	62.26aA	54.19
Topakaşı	51.09aB	53.61bAB	56.81bA	53.84
Mean	49.57	55.11	60.81	**Lsd:3.582**

Each value is expressed as mean ±standard deviation. Means followed by different capital letters (years) in the row are significantly different ($p<0.05$). Means followed by different small letters in the columns (cultivars) are significantly different ($p<0.05$).

The highest amount of oil was obtained at the stage of full ripeness (third harvest period, black purple fruit). The highest average oil ratio (57.46%) was found in Ayvalık. Memecik and Topakaşı had oil contents close to each other (Table 4).
Although the total oil content was highest as percentage in Ayvalık, the obtained dry matter content as above 50% on average in Topakaşı led to the conclusion that this cultivar had the higher oil yield (average 217 g / kg dry fruit flesh) than Ayvalık. In addition, the study results indicated that Topakaşı cultivar, which has completed its dry matter accumulation in the early period, can be harvested at early harvest period (second harvest period: pink-purple) without loss of excess oil yield. On the other hand, it has been concluded that harvesting of Ayvalık and Memecik at the full ripe stage (third period) should be more appropriate in terms of oil yield.
As in other fruit species, especially in table olives, the physical properties of the fruit can vary depending on the cultivar, maturation status and environmental factors. It is possible to see the effects of these factors on olive varieties in previous studies. Likewise, Nas and Gökalp (1990) found the average fruit length, fruit width, fruit/flesh ratio, total dry matter content and total oil content between 17.33-20.62 mm, 12.57-16.09 mm, 61.20%-74.38%, 38.3%-73.0%, 6.0%-24.6%, respectively in a research conducted on some fruit characteristics of some table olive cultivars. Erbay et al. (2010) reported that the fruit width of green olives varied between 13.4 and 16.9 mm. Gümüşoğlu et al. (2006) found that fruit lengths of Domat and Gemlik olive varieties varied between 22.78-27.96 mm and 16.90-23.34 mm, respectively in a research on fruit characteristics of Domat and Gemlik. Kaya and Mutlu (2010) reported that the fruit width, fruit length, fruit/flesh ratio and total oil content were varied between 16.0-19.0 mm, 22.0-24.0 mm, 70%-80%, 6.10%-26.60%, respectively in a research conducted on olives grown in İznik. Özdemir et al. (2011) reported that the fruit/flesh ratio and total oil content were varied between 3.15%-4.87% and 17.53%-32.05%, respectively in a research that aimed to determine the physicochemical changes of olive fruits collected at the different ripening stages. Aşık and Özkan (2011) investigated the fruit characteristics of Memecik olive cultivar and found that the average fruit length, fruit width, seed weight, fruit weight and total oil ratio were 2.55 mm, 1.88 mm, 0.95 g, 5.98 g, 44.74%, respectively.

CONCLUSIONS

The aim of this study was to determine the physical characteristics and total oil of three olive cultivars' fruits at 3 different harvest maturity (skin green with pink spots, pulp white-skin black, skin black and pulp purple) which were grown in Mediterrenean region of Turkey in Isparta/Sütçüler at the same garden and growing conditions.
According to mean values, the highest fruit weight was found in Memecik (4.99 g) followed by Topakaşı (3.49 g) and Ayvalık (3.48 g).

Ayvalık had the lowest (0.65 g) seed weight followed by Memecik (0.76 g) and Topakaşı (0.86 g).
In terms of fruit / seed ratio, Memecik has the best result (84.77%) according to mean values.
The highest amount of dry matter was found in Topakaşı (53.37 g/100 g), followed by Ayvalık (39.00 g/100 g) and Memecik (38.53 g/100 g).
The total amount of oil was highest in Ayvalık (57.46 g/100 g), followed by Memecik (54.19 g/100 g) and Topakaşı (53.84 %).

ACKNOWLEDGEMENTS

The study was supported by the Research Project Coordination Unit under the project number 2601-M-10, at Suleyman Demirel University.

REFERENCES

Al-Maaitah M.I., Al-Absi K.M., Al-Rawashdeh A., 2009. Oil Quality and Quantity of three olive cultivars as influenced by harvesting date in the Middle and Southern Parts of Jordan. Internatıonal Journal of Agriculture &Biology, 11 (3): 266-272.

Aşık H.U., Özkan G., 2011. Physical, chemical and antioxidant properties of olive oil extracted from Memecik Cultivar . Academic Food Journal, 9 (2): 13-18.

Ayton J., Mailer R.J., Haigh A., Tronson D., Conlan D., 2006. Quality and oxidative stability of Australian olive oil according to harvest date and irrigation. J. Food Lipids, 14: 138-156.

Boskou D., 2000. Olive oil. In mediterranean
diets, World. Rev. Nutr. Diet. 56–77.

Erbay B., Üçgül İ., Küçüksayan S., Küçüköner E., 2010. Physical, sensorial, color and rehydration properties of dried green olive slices. Journal of Natural and Applied Sciences, 14 (3): 246-250.

Ercisli, S., Bencic, D., Ipek, A., Barut, E., Liber, Z., 2012. Genetic relationships among olive (*Olea europaea* L.) cultivars native to Croatia and Turkey. Journal of Applied Botany and Food Quality 85: 144 – 149.

Guinda A., Dobarganes M.C., Ruiz-Mendez M.V., Mancha M., 2003. Chemical and physical properties of a sunflower oil with high levels of oleic and palmitic acids. European Journal of Lipid Science and Technology, 105 (3-4): 130-137.

Gümüşoğlu G., İnce A., Güzel E., 2006. Domat ve Gemlik zeytin çeşitlerinde bazı fiziksel özelliklerinin olgunlaşma periyodu süresince değişimi. Tarım Makinaları Bilimi Dergisi, 2(3): 239-244.

Ipek, A., Barut, E., Gulen, H., Ipek, M., 2012. Assessment of inter- and intra-cultivar variations in olive using SSR markers. Sci. Agric., 69(5): 327-335

Kaya Ü., Mutlu T.K., 2010. İznik'te yetiştirilen gemlik zeytininin ve yağının bazı fiziksel, kimyasal ve antioksidan özelliklerinin belirlenmesi. Ç.Ü. Fen Bilimleri Enstitüsü Dergisi, 22(1):199-206.

Keceli T.M., 2013. Influence of time of harvest on 'adana topagi', 'gemlik' olives, olive oil properties and oxidative stability. Journal of Food and Nutrition Research, 1(4): 52-58.

Mousa Y.M., Gerasopoulos D., Metzidakis I., Kiritsaki A., 1996. Effect of altitude on fruit and oil quality characteristics of 'Mastoides' olives. Journal of the Science of Food and Agriculture, 71 (3): 345-350.

Nas S., Gökalp H.Y., 1990. Yusufeli yöresinde üretilen sofralık siyah zeytinlerin bazı fiziksel, kimyasal ve duyusal özellikleri. Gıda, 15 (3): 155-160.

Oktar A., Çolakoğlu A., Işikli T., Acar H.,1983. Zeytinyağı ve teknolojisi. Zeytincilik Araştırma Enstitüsü Yayınları, Bornova-İzmir, 75.

Özdemir Y., Özkan M., Kurultay Ş., 2011. Olgunlaşmayla Gemlik zeytininde oluşan fizikokimyasal değişimler. Bahçe, 40(2): 21-28.

Ryan D., Robards K., 1998. Phenolic compounds in olives. Analyst, 123: 31-44.

Salvador M.D., Aranda F., Gómez-Alonso S., Fregapane G., 2003. Influence of extraction system, production year and area on cornicabra virgin olive oil: a olive oil quality. Eur. J. Lipid Sci. Technol., 104: 602–613.

Servili M., Esposto S., Lodolini E., Selvaggini R., Taticchi A., Urbani S., Montedoro G., Serravalle M., Gucci R., 2007. Irrigation effects on quality, phenolic composition, and selected volatiles of virgin olive oils Cv. Leccino. J. Agric. Food Chem., 55: 6609-6618.

Servili M., Montedoro G., 2002. Contribution of phenolic compounds to virgin olive oil quality. Eur. J. Lipid Sci. Technol., 104: 602–613.

Turaa D., Gigliottib C., Pedoa S., Faillaa O., Bassia D., Serraioccoc A., 2007. Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (*Olea europea* L.) and correlations with oxidative stability. Scientia Horticulturae, 112 (1): 108-119.

Vossen, P.M., 2007. Current Opportunities in the California Olive Oil Industry. Proceedings of the 2007 Plant and Soil Conference of the California Chapter of the American Society of Agronomy, 157-167.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

DETERMINATION OF SOME CHEMICAL PROPERTIES OF 'SWEET ANN' AND 'KABARLA' STRAWBERRY CULTIVARS IN HIGHLAND CLIMATE

Mehmet POLAT[1], Abdullah KANKAYA[2], Mehmet Atilla AŞKIN[1]

[1]Süleyman Demirel University, Faculty of Agriculture, Isparta, Turkey
[2]Elma Tarım LTD Isparta, Turkey

Corresponding author email: mehmetpolat@sdu.edu.tr

Abstract

This study was conducted to determine of some chemical properties of 'Sweet Ann' and 'Kabarla' strawberry cultivars in highland climate. This research was carried out in Isparta region with highland climate conditions. Both varieties of strawberry cultivars used in the research are neutral day plants. Due to the rich polyphenol content of the berries, the positive effects on human health have begun to be explored in recent years. Especially high anthocyanin contents are important. It is well known that the strawberries are rich in polyphenol compounds. In this research, two different production methods were applied, open field and cover cultivation. The total phenolic, total anthocyanin and ascorbic acid content were determined. Total phenolic of 'Sweet Ann' strawberry cultivar varied between 620.44mg GAE 100 g^{-1} FW to 786.64 mg GAE 100 g^{-1} FW. Total anthocyanin of 'Sweet Ann' strawberry cultivar and ascorbic acid ranged between 58.15 µg/g to 98.88 µg/g and 76.42 mg/100 g to 94.39 mg/100 g respectively. Total phenolic of 'Kabarla' strawberry cultivar varied between 448.01 mg GAE 100 g^{-1} FW to 1050.48 mg GAE 100 g^{-1} FW. Also total anthocyanin of 'Kabarla' strawberry cultivar ranged between 47.16 µg/g to 74.44 µg/g. Ascorbic acid content was determined by 125.09 mg/100 g to 134.81 mg/100 g in 'Kabarla' strawberry cultivar. Highest value of total anthocyanin and ascorbic acid were obtained from open field cultivation for both varieties.

Key words: *anthocyanin, ascorbic acid, phenols.*

INTRODUCTION

Having a very important place in human nutrition, strawberry is a rich source of phenolic, anthocyanin and C vitamin. Thanks to the antioxidant substances it contains, this fruit is also beneficial for health. It has been reported by many researchers that the strawberries have the high antioxidant activity. (Cordenunsi et al., 2002; Wang et al., 1996, Cordenunsi et al., 2005).It is important to consume fresh in order to make more use of high antioxidant activity. Because the strawberry is a product that cannot be stored for a long time after being harvested, it will decay immediately. In order to make more use of phenolic substances, vitamin C and anthocyanins found in strawberries, it is necessary to give importance to fresh consumption. Because of the short shelf life, fresh consumption is very important (Cordenunsi et al., 2005). Phenolic substances, vitaminC and anthocyanins, which are very important in terms of health, are affected by environmental factors, harvest maturity, storage conditions as well as by genetic characteristics (Pradas et al., 2015). Hence, this study was conducted to see the effects of two different production methods (open field cultivation and under cover cultivation) on the level of total phenolic, total anthocyanin and ascorbic acid content.

MATERIALS AND METHODS

In this study, "Kabarla" and "Sweet Ann" strawberry varieties were used as plant material. Both are neutral day varieties.
"Kabarla" variety has bears large, hard, sweet fruits with bright red color. It's fruits bearing starts slightly later than the other day variety and it keeps bearing for a long period of time. It bears fruit throughout the summer in highland regions (Anonymous, 2017).
"Sweet Ann" cultivar fruit bears in uplands and passageway regions throughout the summer. It has large, hard, oval and conical shaped bright red colored fruits (Anonymous, 2017). The variety is characterized by vigorous plants

which produce high yields of large to very large. It has sweet fruit with an excellent flavor. And well-shaped, long and conical fruits (Bagdasarian, 2012)

In this research, two different production methods were applied that, open field cultivation and under cover cultivation. Fruits were harvested at the same maturity stage in both varieties. Total phenolic, vitamin-c and anthocyanin were analyzed in fruit samples.

Total phenolic were determined by using the Folin–Ciocalteau reagent according to the method of Singleton and Rossi (1965). Results were explicated as mg GAE 100 g^{-1} FW.

Total anthocyanin was determined by pH differential spectroscopic method (Cheng and Breen, 1991).

Vitamin C was determined spectrophotometrically at 525 nm according to the procedure of Hodges et al. (2001).

The trial was run in triplicate and statistical analysis was done by using the Minitab 17 software package version (Minitab 17 Statistical Software 2010). Differences between means were analyzed by ANOVA test and Tukey test was applied ($P < 0.05$).

RESULTS AND DISCUSSIONS

The total phenolic, anthocyanin and ascorbic acid contents were determined in the "Kabarla" and "Sweet Ann" cultivars grown by two different production methods (Table 1).As you can see from Table 1, the differences between the methods of growing in both varieties in terms of vitamin C are statistically significant. Vitamin C content in "Sweet Ann" cultivars varied from 76.42 to 94.39 mg/100g. Vitamin C content of the “Kabarla” variety was determined between 125.09 mg/100g and 134.81 mg/100g. The "Kabarla" variety has higher vitamin C content in both growing methods than the "Sweet Ann" cultivar. On the other hand, vitamin C content in under cover cultivation is higher than open field cultivation for both varieties.

The amounts of vitamin C in strawberry fruits were reported by Asami et al. (2003), Van de Velde et al. (2013) and Tonutare et al. (2009) as 27.1-32.6 mg/100g, 39.9-44.5 mg/100g and 44-60 mg/100g respectively. Our results are well above these values. And Polat et al. (2016) reported as 94.10-118.87 mg/100g in strawberry fruits. The findings we obtained are consistent with these values.

The total amount of anthocyanin in the "Sweet Ann" variety was found to be 98.88 µg/g in open field and 58.15 µg/g in under cover. In the "Kabarla" varieties, the total anthocyanin content ranged from 47.16 (under cover) to 74.44 µg/g (open field) (Table 1).

In terms of total anthocyanin, the "Sweet Ann" variety has higher values than the "Kabarla" variety. However, total anthocyanin content of open-grown "Kabarla" fruits is higher than "Sweeten" fruits grown under cover (Table 1).

Table 1. Some chemical characteristics of “Sweet Ann” and “Kabarla” cultivars

Cultivar	Growing Methods	Vitamin-C (mg/100g)	Total Anthocyanin (µg/g)	Total Phenolic (mg/100 g FW)
Sweet Ann	Open field	76.42±0.03* b	98.88±0.05* a	786.64±0.52* a
	Under cover	94.39±0.07a	58.15±0.23b	620.44±0.13b
Mean		85.41 B	78.52 A	703.54 B
Kabarla	Open field	125.09±0.03 b	74.44±0.05a	1050.48±0.02 a
	Under cover	134.81±0.02 a	47.16±0.04b	448.01±0.52b
Mean		129,95 a	60,80 B	749,25 A

*The differences between the numbers shown in the same column with different letters are statistically significant (P<0.01)

Polat et al. (2016) are reported that the total amount of anthocyanin as 37.41-105.58 µg/g in fresh strawberry fruits. The data we obtain in our study is in consistent with these values. However, our data are lower than those reported by some other researchers. For example, in a study conducted by Tonutare et al. (2014), the total anthocyanin content was determined as 27.79-60.05 mg/100g in strawberries. In similar researches, Rekika et al. (2005), Zheng et al. (2007) and Wang and Lin (2000) reported that the total amounts of anthocyanin in strawberries are 190.5-841.26 µg/g, 20.07 mg/100g and 38.9 g/100g, respectively. Gill et al. (1997) noticed the total amount of anthocyanin as 113.7-153.5 µg/g. We think that as mentioned by Voca et al. (2014) , the reason of the our findings lower than some of the values reported in the literature, harvesting at different maturity stages is affect the total amount of anthocyanin.

As can be seen in Table 1, in terms of the total phenolic contents, open-grown "Kabarla" fruits showed higher values than the "Sweet Ann" variety. The highest total phenolic value was found in open-grown "Kabarla" fruit (1050.48

mg GAE 100g-1 FW). However, both "Sweet Ann" fruits grown in open field (786.64 mg GAE 100g-1 FW) and under cover (620.44 mg GAE 100g-1 FW) have higher total phenolic content than "Kabarla" fruits grown under cover (448.01 mg GAE 100g-1 FW).

The total amounts of phenolic of strawberry cultivars have reported as 308 to 353 mg/100 g FW by Cordenunsi et al. (2005). Our datas are higher than these values in terms of total phenolic. Polat et al. (2016) reports that, the total phenolic content between 474.97 mg GAE 100g-1 FW and 896.85 mg GAE 100g-1 FW. The findings we obtained in our study are consistent with these values.

CONCLUSIONS

Strawberry fruits have an important place in daily diet nowadays with its rich phenolic substance, anthocyanin and vitamin C content. Some chemical contents of "Kabarla" and "Sweet Ann" varieties have been determined in our research carried out in highland climate conditions. In our research, we tried to reveal the effects of open field cultivation and under cover cultivation on this rich content.

According to the results obtained, we can recommend under cover cultivation to producers and researchers in terms of vitamin C for both varieties. For higher total anthocyanin and total phenolic level, we are recommended the open field cultivation to each two cultivars in highland climate condition.

REFERENCES

Asami D.K., Hong Y.J., Barrett, D.M., Mitchell A.E., 2003. Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices. J. Agric. Food Chem., 51, 1237−1241

Bagdasarian, J. H., 2012. United States Plant. Patent. Patent No: US PP22,472 P3

Cheng G.W., Breen B.J., 1991. Activity of phenylalanyl ammonialyase (PAL) and concentrations of anthocyanins and phenolics in developping strawberry fruit. J. Am. Soc. Hort. Sci. 116, 865–868.

Cordenunsi B.R., Nascimento J.R.O., Genovese, M. I., Lajolo,F. M., 2002. Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. Journal of Agricultural and Food Chemistry, 50, 2581–2586.

Cordenunsi B. R., Genovese, M. I., Nascimento, J. R. O., Mariko, N., Hassimotto, A., Jose´ dos Santos, R. and Lajolo, F. M., 2005. Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. Food Chemistry 91 (2005) 113–121

Gil M. I., Holcroft D.M., Kader A.A., 1997. Changes in Strawberry Anthocyanins and Other Polyphenols in Response to Carbon Dioxide Treatments. J. Agric. Food Chem. (45), 1662−1667

Hodges D.M., Wismer W. V., Forney C.F., 2001. Antioxidant responses in harvested leaves of two cultivars of spinach differing in senescence rates. Journal of the American Society for Horticultural Science, 126, pp. 611–617

Minitab 17 Statistical Software, 2010. State College, PA: Minitab, Inc

Polat, M., Okatan, V., Güçlü, S. F., Çolak, A. M., Korkmaz, N. And Aşkın, M. A., 2016. Effects of GA3 treatments on some chemıcal propertıes of "amıga" and "festıval" strawberry cultıvars. Lucrări Ştiinţifice – vol. 59(2)/2016, seria Agronomie

Pradas, I., Jesús M.J., Víctor O., Manuela M.R.J., 2015. 'Fuentepina' and 'Amiga', two new strawberry cultivars: Evaluation of genotype, ripening and seasonal effects on quality characteristics and health-promoting compounds. Journal of Berry Research, vol. 5, no. 3, pp. 157-171

Rekika,D., Khanizadeh, S., Deschênes, M., Levasseur, A. and Charles, M. T., 2005. Antioxidant Capacity and Phenolic Content of Selected Strawberry Genotypes. HortScience 40(6):1777-1781

Singleton V.L., Rossi J.J.A., 1965. Colorimentry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16 (1965), pp. 144–158

Tõnutare T., Moor, U., Mölder, K., Põldma, P., 2009. Fruit composition of organically and conventionally cultivated strawberry 'Polka'. Agronomy Research 7(Special issue II), 755–760

Tonutare T., Moor, U., Szajdak, L., 2014. Strawberry anthocyanin determination by ph differential spectroscopic method – how to get true results. Acta Sci. Pol., Hortorum Cultus 13(3), 35-47

Wang H., Cao G., Prior R.L., 1996. Total antioxidant capacity of fruits. Journal of Agricultural and Food Chemistry, 44, 701–705.

Wang S.Y., Lin H.S., 2000. Antioxidant Activity in Fruits and Leaves of Blackberry, Raspberry, and Strawberry Varies with Cultivar and Developmental Stage. J. Agric. Food Chem. (48), 140−146

Van De Velde, F., Tarola A.M., Güemes D., Pirovani M.E., 2013. Bioactive Compounds and Antioxidant Capacity of Camarosa and Selva Strawberries (Fragaria x ananassa Duch.) Franco. Foods, 2, 120-131

Voća S., Žlabur J.S., Dobričević N., Jakobek L., Šeruga M., Galić A., Pliestić,S., 2014. Variation in the Bio-active Compound Content at Three Ripening Stages of Strawberry Fruit. Molecules, (19), 10370-10385

Zheng Y., Wang S.Y., Wang C.Y., Zheng W., 2007. Changes in strawberry phenolics, anthocyanins, and antioxidant capacity in response to high oxygen treatments. LWT (40) 49–57.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE DETERMINATION OF OIL PROPERTIES OF SOME OLIVE CULTIVARS GROWN IN SÜTÇÜLER, ISPARTA REGION

Adnan Nurhan YILDIRIM[1], Fatma YILDIRIM[1], Gülcan ÖZKAN[2], Bekir ŞAN[1], Mehmet POLAT[1], Hatice AŞIK[2], Tuba DİLMAÇÜNAL[1]

[1]Suleyman Demirel University, Faculty of Agriculture, Horticultural Science, 32260, Isparta, Turkey
[2]Suleyman Demirel University, Faculty of Engineering, Food Engineering Department, 32260, Isparta, Turkey

Corresponding author email: tubadilmacunal@sdu.edu.tr

Abstract

The aim of this study was to assess the fatty acid compositions, tocopherol contents and some biochemical properties of 'Ayvalık', 'Memecik', and 'Topakaşı' olive cultivars' fruits grown in Mediterrenean region of Turkey, Sütçüler/Isparta. According to mean values the highest value of oleic acid (73.88 %), which is the most dominant acid in olive fruit, was found in Memecik. The highest value for alpha tocopherol content was obtained from Topakaşı (213.63 %) whereas the highest values for beta, gamma and delta tocopherol contenst were obtained from Memecik (2.46, 4.19 and 0.31 % respectively). Memecik had the highest values for chlorophyll (0.47) , carotenoid (0.31) and pheophytin a (2.29) contents according to mean values. According to the knowledge obtained from the research, the fatty acid composition and the quality characteristics of the olive oil are mainly depended on the growing conditions, harvest period and the oil extraction methods. In the study, it is concluded that 2nd harvest period for Memecik, 2nd and 3rd harvest periods for Ayvalık and Topakaşı would be more suitable under the Isparta, Sütçüler growing conditions for high-quality olive oil. This research is the first detailed research on olive in the research area and it is considered that it will be the basis to future scientific studies.

***Key words**: Ayvalık, biochemical properties, fatty acid compositions, tocopherol contents.*

INTRODUCTION

The olive (*Olea europaea* L.) is known the oldest cultivated tree in the world (Özbek, 1975). *Olea europaea* is one of the most important and widespread fruit trees in the Mediterranean basin (Conde et al., 2008), especially in Spain, Italy, Greece and Turkey (Dıraman and Dibeklioğlu, 2009).
Olive oil is an essential constituent of Mediterranean diet and is obtained from the fruit of several cultivars. Each one of these cultivars exhibits specific physical and biochemical characteristics, providing oils with different compositions and performances (Matos et al., 2007). Olive oil is a good source of several bioactive compounds such as mono/poly-unsaturated fatty acids, phenols, phytosterols, carotenoids and tocopherols. Due to their antioxidant properties, these bioactive compounds have reducing effect on the risk of chronic degenerative diseases such as coronary heart disease, cancer, obesity, immune and inflammatory responses (Dag et al., 2015).
Turkey is the sixth largest producer of olive oil in the World (4.6 %) (Sevim et al., 2013) and exported 8% on the average between 2004 and 2010. While the production was 130.000 tonnes in 2008, it increased to 160.000 tonnes in the 2010 (Alkan et al., 2012). The economically important Turkish olive cultivars include Ayvalık, Memecik, Gemlik, Erkence, Nizip Yağlık, and Uslu (Dıraman and Dibeklioğlu, 2009). Olive cultivar 'Memecik' has more than 45% of orchard area in Turkey. Memecik is used both as table olive and for extraction of oil. Olive oil is classified as extra virgin, virgin, olive oil. This classification is carried out according to some quality characteristics (acidity, peroxide value, K_{232}, K_{270}, DK values, alkyl esters) (Caporaso et al., 2015).
The quality of olive oil is affected by many factors such as olive variety, climate and soil characteristics of the geographical region, maturity level, cultural practices and extraction methods (İlyasoğlu and Özçelik, 2011). Olives for oil production are generally harvested in November and December in Turkey. However,

olives are sometimes harvested in early period (October) for production high quality olive oil. So, high quality olive oil was obtained in terms of phenolics and other quality parameters (Yıldırım et al., 2016).

The aim of this study was to determine the variation in the fatty acids, tocopherols and biochemical properties of oils obtained from olive cultivars 'Ayvalık', 'Memecik' and 'Topakaşı' harvested at 3 different maturity stages: (1) early harvest period-1 (green skin with pink spots in less than half of the fruit—Beginning of spotting), (2) early harvest period-2 (pink or purple skin in more than half of the fruit—End of spotting), and (3) optimum harvest period (black skin, less than half of pulp to be purple).

MATERIALS AND METHODS

The study was conducted in the Mediterranean region of Turkey, Sütçüler / Isparta (37° 29'40 "N 30° 58'54" E). In this study, olive cultivars 'Ayvalık', 'Memecik' and 'Topakaşı' grafted on seedling rootstocks were used as materials. Olive trees planted at spacing distance of 5 x 4 m were 10 years old. The altitude of the orchard was 250 m. While Ayvalık and Memecik cultivars are grown in a wide area in Turkey, Topakaşı is only grown in a narrow area of Sütçüler-Isparta, Anamur and Tarsus regions of Turkey. Fruit samples were harvested at 3 different stages of maturity according to the coloring of fruit peel and fruit flesh. These are; (1) Pink spots on green ground (maturity index: 2-3, (2) pink-violet (maturity index: 4-5), and (3) purple-black (maturity index: 6-7).

The obtained natural extra virgin olive oil was placed in dark bottle glasses and kept at -80 °C until analysis. The fatty acid composition of olive oil was determined according to Marquard (1987). 1 ml Na- Methylate (0.5 g Na-methylate + 80 ml methanol + 20 ml iso-octane) solution was added on to 1 ml oil sample and esterification was carried out. 0.25 ml of iso-octane was added before injection and the tube was well-shaken. Then 0.5 ml sample was drawn from the upper phase, which became clear, and by means of a microinjection and injected into the GC apparatus (Pelkin Elmer Autosystem XL). GC condiditions: FID dedector; Cp WAX 52 CB 50 m x 0.32 mm. 1.2 µm column; injector and dedector temperatures: 250^{o}C; carrier gas: He; flow rate: 15 mL/min. The oven temperature was held at 60 ^{o}C for 4 min and increased to 175 with increasing 4 ^{o}C per min. After holding at this temperature for 27 min, the temperature was increased to 215 ^{o}C. After holding at 215 ^{o}C for 5 min, the temperature was gradually increased to 240 ^{o}C. Peaks were identified by taking in to account the relative retention times of standards (Sigma-Aldrich Chemicals 189-19) and results were expressed as percentages of peak areas.

The method of Lampi et al. (1999) was used for tocopherol analysis. Tocopherols (α, γ, δ, and β) were determined by HPLC with a RF-10AXL fluorescence detector (Ex 295-Em 330 nm). 20 µl of the oil was injected into the HPLC device equipped with Luna Silica column (250 × 4.6 mm, 5 µm particle size). Mobile phase: heptane: THF (95:5) (v/v); mobile phase flow rate, 1.2 ml/min; Peak identification was performed according to standards (Cabliochem, Germany). The quantity of tocopherols was calculated according to peak area and expressed as mg kg^{-1} oil.

Analysis of carotenoids and chlorophyll were carried out using spectrophotometer (T70+UV/VIS Spectrometer, PG Instruments Ltd-England). The method defined by Minguez-Mosquera et al. (1991) was used for the determination of carotenoids. 7.5 grams of olive oil was dissolved in cyclohexane and was completed to 25 ml. Samples for total carotenoid were read at 470 nm wavelength and the results were calculated as mg carotenoid/kg fat. The oil samples prepared in cyclohexane were measured at 630 nm, 670 nm and 710 nm wavelengths and the amounts of chlorophyll were calculated as mg of pheophytin a/kg oil using the formula given below (Pokorny et al., 1995).

The amount of chlorophyll (equivalent of mg of pheophytin a / kg fat) = 345.3x [A670-(A630 + A710)] / L

A refers to absorbance, L means the ray path (cell thickness, mm) in the equation.

The method defined by Anonymous (2001) was used for absorbance values. 50 mg of oil sample was weighed and 5% cyclohexane was added to prepare a 1% solution of oil in cyclohexane. The specific absorbance values in UV radiation was determined using K_{232} and K_{270} nm spectrophotometer. The obtained data were analyzed according to One-Way Anova variance analysis method, and significant differences between the averages were determined according to Duncan's test.

RESULTS AND DISCUSSIONS

1. Fatty acid composition

In this study, oleic (71.80-74.19%), palmitic (11.80-13.72%), linoleic (5.92-8.96%) and stearic (2.05- 2.78%) acid were the major fatty acid in the olive oils. Other fatty acids detected in olive oil were tricosanoic, palmitoleic, linolenic, arachidic, gamalinolenic, heptadecaenoic, eicosatrienoic, behenic, heptadecanoic and myristic acid (Table 1). For all fatty acids except for behenic acid, the cultivar x harvest period interaction was found significant. The oleic and linoleic acid contents of the cultivars were differed according to the fruit maturity periods. The content of oleic acid, which is the main acid in olive cultivars, increased significantly with progress of the maturity in Ayvalık, whereas it decreased in Memecik. The highest oleic acid content was found in Ayvalık as 74.19%, followed by Memecik 73.44% and Topakaşı 72.69% at commercial harvest period (third harvest period). Gurdeniz et al. (2008) reported that the oleic acid contents of Ayvalık and Memecik as 69.58 and 66.32%, respectively at commercial harvest period. Dıraman et al. (2009) found that the amount of oleic acid content of Ayvalık changed between 61.44% and 74.68% at different regions and years. Köseoğlu et al. (2016) investigated the oleic acid component of Memecik at 3 stages of ripening according to skin pigmentation as green, purple, and black and found as 72.37%, 71.23% and 68.92%, respectively.

These different results in literature revealed that the growing conditions and harvest time have important effects on the fatty acid compositions and quality attributes of an olive cultivar.

While the content of linoleic acid decreased in Ayvalık, it increased in Memecik with the progress of maturity. According to average values, the highest content of oleic acid among the varieties was determined as 73.88% in Memecik. The cultivars of Ayvalık and Topakaşı had similar contents of oleic acid. While oleic acid was stable in Topakaşı, linoleic acid increased significantly in the third harvest period. In third maturity stage, oleic acid increased while linoleic acid decreased according to average values.

The contents of palmitic acid decreased at significant levels in Ayvalık and Topakaşı with increased maturity. However, no significant difference was obtained in Memecik. The highest average content of palmitic acid was determined as 13.36% in Ayvalık. Similar to our findings, Dıraman et al. (2009) reported that the variation of palmitic and linoleic acids, which are other major fatty acids for Ayvalık, were as 8.94% - 17.77% and 4.68% - 15.14% respectively in different regions and years. In addition, Uğurlu and Özkan (2011) found that the content of palmitic acid in Memecik was 11.38%.

The content of stearic acid in Ayvalık increased significantly in the third harvest period. The palmitic, linoleic and stearic acid contents were found as 12.65%, 5.92% and 2.78%, respectively at the commercial harvest period (third harvest period) (Table 1).

The contents of tricosanoic acid differed according to the harvest periods of the cultivars. Tricosanoic acid increased in Ayvalık with increasing maturity, while it fluctuated in Memecik.

A similar situation was also observed in Topakaşı. The results of the study showed an increase in palmitoleic acid content increasing maturity. The highest values were determined during the third harvesting period in all cultivars. The highest palmitoleic acid content was determined in Ayvalık with an average of 1.12%, followed by Topakaşı (0.83%) and Memecik (0.79%). Similar to our result, Uğurlu and Özkan (2011) found that palmitoleic acid content was 0.50% in Memecik. The content of linolenic acid increased in all cultivars with incresing maturity. The highest average linolenic acid content was found in Memecik as 0.65%, followed by Ayvalık as 0.54%. Tanılgan et al. (2007) found the linolenic acid content as 0.2% in Ayvalık.

Table 1. Composition of fatty acids (%) obtained from Ayvalık, Memecik and Topakaşı olive cultivars in three different harvesting periods.

Olive cultivars	First harvest	Second harvest	Third harvest	Mean Values
Oleic acid (C18:1)				
Ayvalık	71.80bB	71.93bB	74.19aA	72.64
Memecik	74.72aA	73.48aB	73.44bB	73.88
Topakaşı	72.29b	72.88a	72.69c	72.62
Mean	72.93	72.76	73.44	**Lsd:0.7012**
Palmitic acid (C16:0)				
Ayvalık	13.72aA	13.70aA	12.65B	13.36
Memecik	11.80b	12.01c	12.28	12.03
Topakaşı	13.59aA	12.96bB	12.44C	12.99
Mean	13.03	12.89	12.46	**Lsd:0.4984**
Linoleic acid (C18:2)				
Ayvalık	8.96aA	8.01bB	5.92cC	7.63
Memecik	7.41cC	8.75aA	8.26bB	8.14
Topakaşı	8.27bB	8.24bB	8.76aA	8.42
Mean	8.21	8.33	7.65	**Lsd:0.4416**
Stearic acid (C18:0)				
Ayvalık	2.46aB	2.38aB	2.78aA	2.54
Memecik	2.18bA	2.05bB	2.09cAB	2.11
Topakaşı	2.28b	2.33a	2.39b	2.33
Mean	2.31	2.25	2.42	**Lsd:0.1168**
Tricosanoic acid (C23:0)				
Ayvalık	0.77cC	0.94B	1.13aA	0.94
Memecik	1.28aA	0.93C	1.14aB	1.11
Topakaşı	0.90b	0.94	0.90b	0.91
Mean	0.98	0.93	1.05	**Lsd:0.08924**
Palmitoleic acid (C16:1)				
Ayvalık	0.85aB	1.21aA	1.32aA	1.12
Memecik	0.71bB	0.81bAB	0.85bA	0.79
Topakaşı	0.77ab	0.83b	0.89b	0.83
Mean	0.78	0.95	1.02	**Lsd:0.1282**
Linolenic acid (C18:3)				
Ayvalık	0.46bB	0.49bB	0.67aA	0.54
Memecik	0.54aB	0.68aA	0.72aA	0.65
Topakaşı	0.47bAB	0.44bB	0.51bA	0.47
Mean	0.49	0.54	0.63	**Lsd:0.06176**
Arachidic acid (C20:0)				
Ayvalık	0.33bC	0.39B	0.46A	0.39
Memecik	0.44a	0.41	0.45	0.43
Topakaşı	0.46a	0.43	0.44	0.44
Mean	0.41	0.41	0.45	**Lsd:0.04317**
Gamma-linolenic acid (C18:3)				
Ayvalık	0.22bC	0.30bB	0.38aA	0.30
Memecik	0.38a	0.37a	0.41a	0.38
Topakaşı	0.36a	0.33b	0.35b	0.34
Mean	0.32	0.33	0.38	**Lsd:0.03939**
Heptadecanoic acid (C17:0)				
Ayvalık	0.18B	0.21aA	0.19bAB	0.19
Memecik	0.17A	0.15bA	0.09cB	0.13
Topakaşı	0.18B	0.23aA	0.23aA	0.21
Mean	0.18	0.19	0.17	**Lsd:0.02659**
Eicosatrienoic acid (C20:1)				
Ayvalık	0.10cB	0.18aA	0.08bC	0.12
Memecik	0.24aA	0.18aB	0.06cC	0.16
Topakaşı	0.18bA	0.13bB	0.18aA	0.16
Mean	0.17	0.16	0.11	**Lsd:0.01174**
Behenic acid (C22:0)				
Ayvalık	0.12	0.15	0.13	0.13
Memecik	0.12	0.13	0.14	0.13
Topakaşı	0.13	0.14	0.13	0.13
Mean	0.12B	0.14A	0.13AB	**Lsd:0.01509**
Heptadecenoic acid (C17:1)				
Ayvalık	0.04bB	0.13aA	0.12aA	0.09
Memecik	0.04bB	0.07bA	0.07bA	0.06
Topakaşı	0.14aA	0.13aA	0.10aB	0.12
Mean	0.07	0.11	0.09	**Lsd:0.02176**
Myristic acid (C14:0)				
Ayvalık	0.010cB	0.020A	0.010bB	0.013
Memecik	0.017bB	0.020A	0.020aA	0.019
Topakaşı	0.020a	0.020	0.020a	0.020
Mean	0.016	0.020	0.020	**Lsd:0.002998**

Each value is expressed as mean ±standard deviation. Means followed by different capital letters (years) in the row are signifi cantly different ($p<0.05$). Means followed by different small letters in the columns (cultivars) are signifi cantly different ($p<0.05$).

Our findings are similar those of İlyasoğlu and Özçelik (2011) in terms of palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acid components of Memecik.

The arachidic and gamma-linolenic acid contents increased only in Ayvalık at the significant level with increasing maturity. However, there was no significant difference between the harvest periods for the other two cultivars. The highest average arachidic acid was found in Topakaşı and the highest gamma-linolenic acid was found in Memecik. Dıraman and Dibeklioğlu (2014) found similar results in terms of arachidic acid contents of Ayvalık and Memecik.

In terms of heptadecanoic acid content, there was an increase in Topakaşı in the second harvest period and this increase remained constant during the third harvest period. On the other hand, Ayvalık showed an increase in terms of heptadecanoic acid in the second harvest period and but it was decreased in the third harvest period. The content of heptadecanoic acid decreased in Memecik with increasing maturity and it decreased at significant level in the third harvest period. The highest average heptadecanoic content was determined in Topakaşı as 0.21%, followed by Ayvalık as 0.19%.

Eicosatrienoic acid content increased significantly at the second harvest period in Ayvalık and Topakaşı. the content of eicosatrienoic acid showed a decreasing at significant level in Memecik with progress of maturity. The highest eicosatrienoic content was found in Memecik and Topakaşı as 0.16%.

There was no significant difference between cultivars and harvest periods in terms of behenic acid content.

In terms of heptadecanoic content, significant increases observed in the second harvest period in Ayvalık and Memecik and this increase remained stable in the third harvest period. On the contrary, a significant decrease was observed in Topakaşı in the third harvest period. The highest average content of heptadecanoic acid was determined in Topakaşı as 0.12%.

While the content of myristic acid increased during the second harvest period, it decreased in third harvest period in Ayvalık. The fatty acid components of Ayvalık and Memecik obtained in this research were similar with those of Dıraman (2010) and Dıraman and Dibeklioğlu (2014).

2. Tocopherol contents

One of the most important minor chemical components in olive oils is tocopherols. Beside their health benefits, they also enhance the oxidative stability of olive oils due to their antioxidant properties (Dag et al., 2015). In terms of all tocopherol components, the cultivar x harvest period interaction was found significant.

Among the different tocopherol forms, alpha (α) tocopherol was the major one in all cultivars. The highest α-tocopherol contents were determined in the second harvest period in Ayvalık and Memecik while the highest value was found in the third harvest period in Topakaşı.

The highest average α-tocopherol content was determined in Topakaşı (213.63 mg/kg oil), followed by Ayvalık (212.97 mg/kg oil) (Table 2). Beta (β) tocopherol content increased significantly during the second harvest period in all cultivars and decreased to the lowest level in the third harvest period. The highest average content of β-tocopherol was determined in Memecik (2.46 mg/kg oil). While the content of gamma (γ) tocopherol showed an increasing in Ayvalık with increasing maturity, a decreasing was found in Topakaşı. It increased at second harvest period and then decreased at third harvest period in Memecik.

The highest average content of γ-tocopherol was determined in Memecik (4.19 mg/kg oil), followed by Ayvalık (2.31 mg/kg oil). The highest delta (δ) tocopherol content was determined in the first harvest period in Ayvalık and Topakaşı. The highest average δ-tocopherol content was found in the Memecik as 0.31 mg/kg oil (Table 2). Uğurlu and Özkan (2011) reported that the values of α, β, γ and δ-tocopherol were 205.45 mg/kg oil, 1.645 mg/kg oil, 6.065 mg/kg oil and 0.325 mg/kg oil, respectively in Memecik. These results are similar to our average results obtained in Memecik.

The highest K_{232} values were determined in the second harvest period in all cultivars and the lowest values were determined in the third harvest period. The highest average K_{232} value was determined as 1.57 in the Ayvalık while the lowest value was 1.38 in Memecik (Table 3). The highest average K_{270} value was determined as 0.14 in Ayvalık. Similar to our results, oil quality parameters of K_{232} and K_{270} were found as 1.493 and 0.098, respectively in Memecik by Uğurlu and Özkan (2011).

Table 2. Composition of tocopherol obtained from Ayvalık, Memecik and Topakaşı olive cultivars in three different harvest periods.

Olive cultivars	First harvest	Second harvest	Third harvest	Mean Values
	Alpha (α) tocopherol (mg/kg oil)			
Ayvalık	186.85cC	229.65aA	222.40bB	212.97
Memecik	205.50bB	215.25bA	200.25cC	207.00
Topakaşı	210.15aB	201.30cC	229.45aA	213.63
Mean	200.83	215.40	217.37	**Lsd:3.581**
	Beta (β) tocopherol (mg/kg oil)			
Ayvalık	1.57cB	1.77aA	1.42bC	1.59
Memecik	2.45aB	2.76aA	2.17aC	2.46
Topakaşı	1.72bB	1.79bA	1.27cC	1.59
Mean	1.91	2.10	1.62	**Lsd:0.01341**
	Gamma (γ) tocopherol (mg/kg oil)			
Ayvalık	1.64bC	2.44bB	2.87bA	2.31
Memecik	3.67aB	5.28aA	3.63aC	4.19
Topakaşı	1.64bA	1.56cB	1.14cC	1.45
Mean	2.32	3.09	2.54	**Lsd:0.02448**
	Delta (δ) tocopherol (mg/kg oil)			
Ayvalık	0.13cA	0.11bB	0.10bC	0.11
Memecik	0.24aC	0.42aA	0.28aB	0.31
Topakaşı	0.17bA	0.07cC	0.09cB	0.11
Mean	0.18	0.19	0.16	**Lsd:0.004240**

Each value is expressed as mean ±standard deviation. Means followed by different capital letters (years) in the row are signifi cantly different ($p<0.05$). Means followed by different small letters in the columns (cultivars) are signifi cantly different ($p<0.05$).

Table 3. Some biochemical properties of cultivars

Olive cultivars	First harvest	Second harvest	Third harvest	Mean Values
	Chlorophyll (mg/kg oil)			
Ayvalık	0.31cA	0.24cB	0.17cC	0.24
Memecik	0.52aA	0.43aB	0.46aB	0.47
Topakaşı	0.37bA	0.32bB	0.22bC	0.30
Mean	0.40	0.33	0.28	**Lsd:0.02665**
	Carotenoid (mg/kg oil)			
Ayvalık	0.26cA	0.23cB	0.20bC	0.23
Memecik	0.35aA	0.28aB	0.29aB	0.31
Topakaşı	0.29bA	0.26bB	0.18cC	0.25
Mean	0.30	0.26	0.22	**Lsd:0.008654**
	Pheophytin-a (mg/kg oil)			
Ayvalık	1.08cA	0.74cB	0.34cC	0.72
Memecik	2.69aA	2.04aC	2.13aB	2.29
Topakaşı	1.46bA	0.90bB	0.46bC	0.94
Mean	1.74	1.23	0.98	**Lsd:0.01731**
	K_{232}			
Ayvalık	1.58aA	1.59aA	1.56aB	1.57
Memecik	1.40cA	1.41bA	1.35cB	1.38
Topakaşı	1.51bB	1.58aA	1.50bB	1.53
Mean	1.49	1.53	1.47	**Lsd:0.01095**
	K_{270}			
Ayvalık	0.15aA	0.13aB	0.15aA	0.14
Memecik	0.12bA	0.11bAB	0.10cB	0.11
Topakaşı	0.12b	0.13a	0.12b	0.13
Mean	0.13	0.13	0.13	**Lsd:0.01187**

Each value is expressed as mean ±standard deviation. Means followed by different capital letters (years) in the row are signifi cantly different ($p<0.05$). Means followed by different small letters in the columns (cultivars) are signifi cantly different ($p<0.05$).

İlyasoğlu and Özçelik (2011) also found similar result to our finding in term of K_{270} value. In this research, the interaction between chlorophyll, carotenoid and pheophytin-a contents and harvest periods were found significant. Chlorophyll, carotenoid and pheophytin-a contents decreased significantly with increasing maturity. The highest average values of chlorophyll, carotenoid, and pheophytin-a were determined in Memecik (0.47, 0.31 and 2.29, respectively) (Table 3). Our findings are similar to those of Uğurlu and Özkan, (2011) which reported the average value of chlorophyll content of Memecik as 0.49 mg/kg of oil.

CONCLUSIONS

In this research, the effects of cultivar and harvest period on the fatty acid composition were significantly determined.

According to the knowledge obtained from the research, the fatty acid composition and the quality characteristics of the olive oil are mainly depended on the growing conditions, harvest period and the oil extraction methods.

In the study, it is concluded that 2nd harvest period for Memecik, 2nd and 3rd harvest periods for Ayvalık and Topakaşı would be more suitable under the Isparta/Sütçüler growing conditions for high-quality olive oil.

ACKNOWLEDGEMENTS

The study was supported by the Research Project Coordination Unit under the project number 2601-M-10, at Suleyman Demirel University.

REFERENCES

Alkan D., Tokatli F., Ozen B., 2012. Phenolic characterization and geographical classification of commercial extra virgin olive oils produced in Turkey. J. Am. Oil Chem. Soc. 89: 261–268.

Caporaso N., Savarese M., Paduano A., Guidone G., De Marco E., Sacchi R., 2015. Nutritional quality assessment of extra virgin olive oil from the Italian retail market: Do natural antioxidants satisfy EFSA health claims? Journal of Food Composition and Analysis 40: 154–162.

Anonymous, 2001. Codex Alimentarius Commission. Codex Standard 12-1981, Rev. 2.

Conde C., Delrot S., Geros H., 2008. Physiological, biochemical and molecular changes occuring during olive development and ripening. J. Plant Physiol. 165, 1545-1562.

Dag C., Demirtas I., Ozdemir I., Bekiroglu S., Ertas E., 2015. Biochemical characterization of Turkish extra virgin olive oils from six different olive varieties of ıdentical growing conditions. J. Am. Oil Chem. Soc. 92:1349–1356.

Dıraman H., Dibeklioğlu H., 2009. Characterization of Turkish virgin olive oils produced from early harvest olives. J. Am. Oil Chem. Soc. 86:663–674.

Dıraman H., Özdemir D., Hışıl Y., 2009. Characterization of early harvest virgin olive oils produced from ayvalık cultivar based on their fatty acid profiles by chemometrics. Electronic Journal of Food Technologies 4 (3): 1-11.

Dıraman H., 2010. Characterization by chemometry of the most important domestic and foreign olive cultivars from the national olive collection orchard of Turkey. Grasas y Aceites, 61 (4): 341-351.

Dıraman H., Dibeklioglu H., 2014. Using lipid profiles for the characterization of Turkish monocultivar olive oils produced by different systems. International Journal of Food Properties, 17: (5): 1013-1033.

Gurdeniz G., Ozen B., Tokatli F., 2008. Classification of Turkish olive oils with respect to cultivar, geographic origin and harvest year, using fatty acid profile and mid-IR spectroscopy. Eur. Food Res. Technol. 227: 1275–1281.

İlyasoğlu H., Özçelik, B.B, 2011. Biochemical characterization of Memecik olive oils. Food, 36 (1): 33-41.

Köseoğlu O., Sevim D., Kadiroğlu P., 2016. Quality characteristics and antioxidant properties of Turkish monovarietal olive oils regarding stages of olive ripening. Food Chemistry 212: 628–634.

Lampi A.M., Kataja L., Kamal-Eldin A., Vieno P., 1999. Antioxidant activities of α- and γ- tocopherols in the oxidation of rapeseed oil triacylglycerols. Journal of the American Oil Chemists' Society, 76 (6): 749-755.

Marquard R., 1987. Qualitatsanalytik im dienste der ölpflanzenzüchtung. Fat.Sci. Technol. 89: 95-99.

Matos L.C., Pereira J.A., Andrade P.B., Seabra R.M., Oliveira M.B.P.P., 2007. Evaluation of a numerical method to predict the polyphenols content in monovarietal olive oils. Food Chemistry 102, 976–983.

Minguez-Mosquera M.I., Rejano-Navarro L., Gandul-Rojas B., Gomez A.H.S., Garrido-Fernandez J., 1991. Color- pigment correlation in virgin olive oil. Journal of the American Oil Chemists Society, 68 (5): 332-336.

Özbek S., 1975. General fruit growing. Çukurova University Agricultural Faculty Publications, 111. Textbook: 6, p.386, Ankara.

Pokorny J., Kalinova L., Dysseler P., 1995. Determination of chlorophyll pigments in crude vegetable oils: Results of a collaborative study and the standardized method (technical report). Pure and Applied Chemistry, 67 (10): 1781–1787.

Sevim D., Köseoğlu O. Öztürk G.F., 2013. Effect of different growing area on triacylglycerol composition of cv. Gemlik olive oil in Turkey.

Journal of Agricultural Faculty of Uludag University, 27 (1): 49-54.
Tanılgan K., Özcan M.M., Ünver A., 2007. Physical and chemical characteristics of five Turkish olive (Olea europea L.) varieties and their oils. Grasas y Aceites, 58 (2): 142- 147.
Uğurlu A.H., Özkan, G., 2011. Physical, chemical and antioxidant properties of olive oil extracted from Memecik cultivar. Academıc Food Journal 9 (2): 13-18.
Yıldırım F., Yıldırım A.N., Özkan G., Şan B., Polat M., Aşık H., Karakurt Y., Ercişli S., 2016. Early harvest effects on hydrophilic phenolic components of extra virgin olive oils cvs. 'Ayvalık,' 'Memecik,' and 'Topakaşı'. Biochem. Genet., DOI 10.1007/s10528-016-9784-3.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PHYSICAL AND BIOCHEMICAL CHANGES IN POMEGRANATE (*PUNİCA GRANATUM* L. cv. 'HİCAZNAR') FRUITS HARVESTED AT THREE MATURITY STAGES

Berna BAYAR, Bekir ŞAN

Suleyman Demirel University, Faculty of Agriculture, Horticultural Science, 32260, Isparta, Turkey

Corresponding author email: bekirsan@sdu.edu.tr

Abstract

This study was carried out to determine the physical and biochemical changes in fruits harvested at three different maturity stages in pomegranate cultivar 'Hicaznar'. Fruits were harvested at the periods of 1) the time of the beginning of the color change in arils (August 15), 2) the time of the pink color of arils (September 6) and 3) the time of the red color of arils (October 3). In the study, fruit weight, fruit juice, colour (L, a*, b*), titratable acidity, pH, total soluble solids, total phenolics, phenolic composition (gallic, chlorogenic, ellagic and syringic acids) and organic acids (malic and citric acids) were investigated. Significant increases in fruit weight, total soluble solids, malic acid and colour value of a* were detected with progress of maturity. On the contrary, titratable acidity, total phenolics, gallic, ellagic and sitric acids were significantly decreased with progress of maturity. The changes in chlorogenic and syringic acids were not statistically significant.*

Key words: *pomegranate, maturity, phenols, organic acids.*

INTRODUCTION

Pomegranate belongs to the *Punica* genus of Punicaceae family, the most important species being *Punica granatum* L. Pomegranate is one of the oldest known fruit species and its cultural history dates back to around 3000 BC. It can grow up to 1000 m above sea level in tropical and subtropical climates. Pomegranate is cultivated widely in the Mediterranean Basin, South West Asia and America in the world. Pomegranate plants have many advantages such as being easily adaptable to various climatic and soil conditions, being easy to replicate and having high productivity. Pomegranates are also grown as ornamental plants or hedge plants in Turkey. Pomegranate production, which is 59 000 tonnes in 2000 in Turkey, reached 445 750 tonnes in 2015 with a big increase. Pomegranate is a very rich fruit species in terms of phenolic substances, flavonoids, tannins, fatty acids, aromatic compounds, amino acids, tocopherols, sterols and terpenoids (Ozgen et al., 2008; Wang et al., 2010).

In addition to many factors such as increasing environmental pollution, ultraviolet rays and smoking, stressful living conditions, environmental and psychological factors negatively affect human health and cause free radicals formation. It has became important to consume natural nutrients instead of using drugs to remove the negative effects of free radicals and to prevent disease formation (Hochstein and Atallah, 1988; Benzie, 2003). Fruits that are rich in terms of phenolic substances prevent these diseases by preventing these free radicals and strengthen the immune system. In addition to that, they have positive effects on health due to their antimicrobial and antioxidative effects. The most important factor affecting the nutritional content of fruits evaluated as functional food is genotype. However, it is a known fact that environmental conditions also influence the nutritional contents of fruits. Researchers have reported that the antioxidant capacities of fruits vary significantly with respect to fruit maturity levels as well as ecological (temperature, soil characteristics and night-time temperature difference) and cultural (irrigation, fertilization) conditions (Gao et al., 2012). There are many studies interested in the increase of the nutrient contents of fruits by different applications (Rossi et al., 2003, Ancos et al., 2000). It has been reported that the nutritional content of fruit species changes significantly with maturity level. For example,

in a study of two different species of *Zizipus*, it was determined that the amounts of phenolic compounds in green fruits were higher than those in ripe ones in *Z. mauritiana* and *Z. nummularia* species (Choi et al., 2012; Wu et al., 2012).
The objective of this study was to evaluate the pomegranate fruit harvested at different maturity levels in terms of their physical and biochemical contents.

MATERIALS AND METHODS

Materials

In the study, the fruits harvested in three maturity levels of pomegranate cultivar Hicaznar were used. The fruits were obtained from a farmer's orchard in Serik region of Antalya, Turkey.

Methods

In the study, fruits were harvested at 3 different maturity levels. In this regard, pomegranate fruits were harvested at 1) the time of the beginning of the color change in arils (August 15), 2) the time of the pink color of arils (September 6) and 3) the time of the red color of arils (commercial harvest time, October 3).
Determination of physical properties. Fruit width, fruit height, fruit weight, peel weight and aril weight were determined in the harvested fruit. Peel color of fruits were measured using a colorimeter (Chroma Meter CR-400, Minolta) and expressed as L*, a* and b* values.
Determination of biochemical properties. In order to determine the biochemical properties, the arils separated from their peels were squeezed and the obtained fruit juice was filtered through filter paper. The pH of the fruit juice was determined by a pH meter (Hanna). Total soluble solids were measured with a hand refractometer and expressed as %. Titratable acid content was determined according to the method described by Karaçalı (1990) and calculated as % citric acid.
The total phenolic content of fruit juices was determined using the Folin-Ciocalteu method. For this purpose, the aril juice was diluted 1:5 with etanol. 100µl of fruit juice was added and 3ml of purified water was added. Then, 200 µL of Folin-Ciocalteu (0.2N) and 100 µL of sodium carbonate (20%) were added and incubated in the dark for 2 hours. The absorbance values were then read on a spectrophotometer adjusted to a wavelength of 765 nm. The total amount of phenolic substances in pomegranate juice was calculated from the standard calibration curve. For the determination of standard calibration curve, 50, 100, 150, 200, 250, 300, 350 and 400 mg/L gallic acid solutions were prepared and their absorbances were read at 765 nm in a spectrophotometer by the same method.
Phenolic contents were analyzed according to the modified procedure of Caponio et al. (1999). The 5 ml of fruit juice was mixed with 10 ml of methanol (80%). The sample was incubated in an ultrasonic bash for 10 min and centrifuged at 4000 rpm for 10 min. The upper phase was filtered through a 0.45 µm membrane filter (Millipore) and 20µl of the sample was injected into an HPLC (Shimadzu Inc) equipped with a diode array detector (lmax = 278), Agilent Eclipse XDB-C18 column (250x4,6 mm, 5µm) operated at 30^{o}C, a SIL-10AD vp autosampler, a LC-10AD vp pump, a CTO-10Avp column oven, and a DGU-14A degasses. The mobile phase consisted of 3% acetic acid (A) and methanol (B). The flow rate was 0.8 mL/min. The gradient program was given in Table 1. The peaks were identified by comparison with the peak of standard of gallic acid, catechine, chlorogenic acid, vanillic acid, syringic acid, ellagic acid, quercetin and kaempherol (Sigma Chemical Co) (Figure 1). The phenolics were expressed as µg per g fruit juice.

Table 1. The linear solvent gradient system used in HPLC analysis of phenolics.

Time (min)	**0.1**	**20**	**28**	**35**	**50**	**60**	**62**	**70**	**73**	**75**	**80**	**81**
A *(%)	93	72	75	70	70	67	58	50	30	20	0	93
B (%)	7	28	25	30	30	33	42	50	70	80	100	7

*Solvent A: 3% Acetic acid, Solvent B: Methanol

Table 2. Some physical properties of fruits of pomegranate cultivar 'Hicaznar' harvested at 3 different periods

Harvest date	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Shape index	Peel weight (g)	Aril weight (g)	Aril yield (%)
August 15	260.8 c*	70.7 c	79.5 c	1.1	131.6 c	129.1 c	0.49
September 6	326.9 b	76.7 b	86.7 b	1.1	172.6 b	154.2 b	0.47
October 3	438.5 a	87.9 a	97.2 a	1.1	235.3 a	203.2 a	0.46

* Means followed by different letters in the same column are significantly different from each other (p≤0.05).

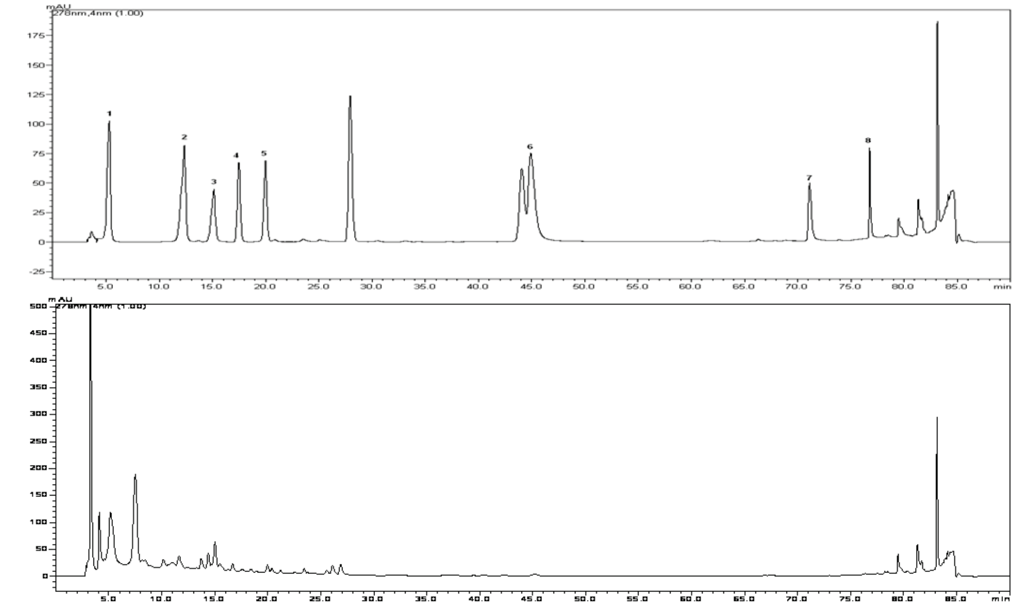


Figure 1: HPLC chromatograms of phenolic standards and extracts of pomegranate fruit juice. 1; Gallic acid 2; Catechin 3; Chlorogenic acid 4; vanilic acid 5; syringic acid, 6; ellagic acid, 7; quercetin 8; Kaempferol

Organic acid contents of pomegranate juice were analized according to the modified procedure of Alhendawi et al. (1997) and Kordis-Krapez et al. (2001). 2 ml of fruit juice was diluted with 2 ml H_3PO_4 (2%) and then 1ml of sample was diluted with 1 ml of extraction solution (0.01M KH_2PO_4, pH: 8.0). 20µl of sample was injected into an HPLC (Shimadzu Inc) equipped with SPD-10Avp UV-VIS detector (210nm), SIL-20AC prominence auto sampler, LC-20AT prominence system controller, LC-20AT prominence Pump, DGU-20A5 degasser and Prodigy ODS-2 (250x4.6mm, 5µm) column operated at 30^{o}C. The mobile phase was distiled water adjusted to pH 2.25 with phosphoric acid. The flow rate was 0.8 mL/min. Peaks were identified by comparison with the peak of standard of tartaric, malic, ascorbic, citric and sucsinic acids (Sigma Chemical Co) (Figure 2). The organic acids were expressed as µg per g fruit juice.

Data analysis: The experiment was planned according to a completely randomized design with three replications. The data were subjected to the analysis of variance using the MINTAB software (MINITAB Inc.) and the means were separated from each other by Tukey's test at the 5 % level of significance.

RESULTS AND DISCUSSION

Fruit weight, fruit height, fruit width, peel weight and aril weight values of pomegranate cultivar 'Hicaznar' fruits harvested in 3 different periods increased significantly with maturity. Fruit shape index and aril yield were not changed significantly by maturity stages in the study (Table 2). The results obtained in the study were found to be similar to those of Özsayın (2012). It has been determined that the fruit weight increased about 2 times in the last 48 days (from 15 August to 3 October).

Table 3. Changes in values of fruit peel color of pomegranate cultivar 'Hicaznar' harvested at 3 different periods

Harvest Date	L^*	a^*	b^*
August 15	55.88 b*	-1.28 c	33.18
September 6	59.47 a	13.31 b	33.09
October 3	58.04 ab	30.49 a	31.39

* Means followed by different letters in the same column are significantly different from each other (p≤0.05).

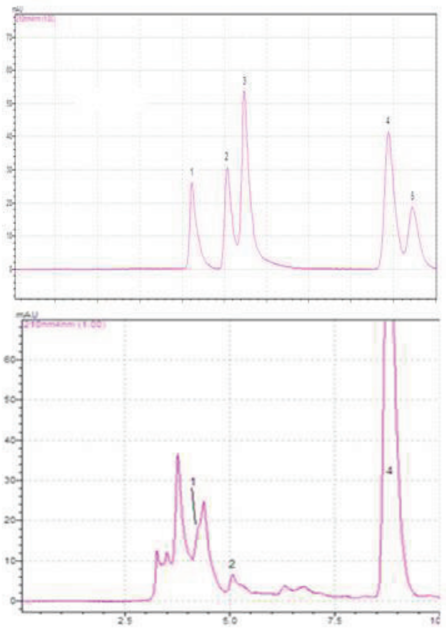


Figure 2: HPLC chromatograms of organic acid standards and extracts of pomegranate fruit juice. 1; tartaric acid 2; malic acid 3; ascorbic acid 4; sitric acid 5; succinic acid

With regard to the fruit peel color, the b* value did not change while a significant increase in a* value was determined with maturity. A relative increase was observed with maturity in terms of L* value (Table 3). It has been determined that the highest pH value (2.77) of the fruit juice was in the commercial harvesting period (October 3). Correspondingly, the content of titratable acidity has also decreased regularly with the progress of the maturity. As expected, the total soluble solid content of juices increased steadily with the progress of the maturity and this increase was found to be statistically significant. The content of total soluble solid was found to be 15.7% for the fruits harvested in the commercial harvesting period. Similar to our findings, Kulkarni and Aradhya (2005) reported that the content of total soluble solids increased, while the content of titretable acidity decreased with the progress of the maturity in pomegranate. In another study, it was reported that the total soluble solid content of pomegranate cultivar 'Hicaznar' varied between 14% and 18.2% in the commercial harvest time (Özsayın, 2012), which was similar to our results. The malic acid content of fruits significantly increased with the progress of the maturity. While malic acid content was 317.2 µg/g on August 15, this value increased to 545.2 µg/g at commercial harvest date (October 3). The citric acid content of the fruit was 25323 µg/g on August 15 and decreased by half to 12666 µg/g at the time of commercial harvest (October 3). Tartaric acid was not found in the fruits harvested on August 15 and September 6, but was found to be 466.8 µg/g on October 3 (Table 4). The contents of malic, sitric and tartaric acids of fruits observed in our study were similar to those reported by Karaca (2011). Karaca (2011) reported that sitric acid, malic acid and tartaric acid contents of mature fruit were 17360 µg/g, 500 µg/g and 590 µg/g, respectively. It has also been reported that the content of organic acids in myrtle decreases with maturity (Mulas et al., 2013).

The total phenolic contents of 'Hicaznar' fruit juice were found to be higher in the immature fruit than in the mature fruit. In the study, the total phenolic content in fruit juice was the highest (8308 ± 335 µg / g) at the beginning of coloring (15 August) and decreased significantly with maturity. Similarly, the amounts of gallic acid and ellagic acid in the fruit juice were found to be the highest (97.2 and 13.6 µg/g, respectively) in fruits harvested on August 15 and were reduced to 29.47 and 4.46 µg/g, respectively in fruits harvested on October 3 (commercial harvest date). There were no statistically significant differences in the fruits harvested at the different maturity stages in terms of chlorogenic and syringic acids (Table 5). In support of our results, Al-Maiman and Ahmad (2002) found that the total phenolic amount of pomegranate fruits decreased significantly with the progress of maturity.

Table 4. Changes in pH, total soluble solid, titretable acidity and orhanic acids of pomegranate cultivar 'Hicaznar' harvested at 3 different periods

Harvest Date	pH	Total Soluble Solid (%)	Titretable Acidity (%)	Malic acid (µg/g)	Sitric acid (µg/g)	Tartaric acid (µg/g)
August 15	2.70 ab*	10.93 b	3.07 a	317.2 b	25323 a	-
September 6	2.57 b	11.76 b	2.49 ab	354.6 b	16271 ab	-
October 3	2.77 a	15.70 a	1.95 b	545.2 a	12666 b	466.8

* Means followed by different letters in the same column are significantly different from each other ($p \leq 0.05$).

Table 5. Changes in phenolic compounds of pomegranate cultivar 'Hicaznar' harvested at 3 different periods

Harvest Date	Total phenolics (µg/g)	Gallic Acid (µg/g)	Chlorogenic Acid (µg/g)	Syringic Acid (µg/g)	Ellagic Acid (µg/g)
August 15	8308 a*	97.2 a	83.9	5.7	13.6 a
September 6	5896 b	50.8 ab	84,2	6.3	4.8 b
October 3	5696 b	29.5 b	66.3	5.0	4.5 b

* Means followed by different letters in the same column are significantly different from each other ($p \leq 0.05$).

Similarly, Siriamornpun et al. (2015) reported that immature green fruits contained more total phenolic substance than mature fruits in jujube. Moreover, it was reported that total phenolic, gallic acid and ellagic acid contents of myrtle decreased with the progress of maturity which was similar to our results (Fadda and Mulas, 2010; Babou et al., 2016). On the other hand, unlike our findings, it was reported that gallic acid content did not change with the progress of maturity in 'Hicaznar' (Özhan-Tümer, 2006). It has been found that the content of flovonols in *Morus alba* species generally decreases with maturity (Lee and Choi, 2012), but not in *Vitis vinifera* species (Doshi et al., 2006). It has been reported in many studies that the amounts of phenolic substances in fruit species may vary significantly according to genotype, ecological conditions and analysis method (Karaca, 2011; Wang et al., 2010).

CONCLUSIONS

As a result, the biochemical contents of the 'Hicaznar' fruits vary significantly with their maturity level. Malic acid content increases with maturity, while citric acid content decreases. Tartaric acid was not detected in immature fruit. It has been found that the total phenolic substance, gallic acid and ellagic acid contents decrease with maturity. Fruits should still be harvested at commercial harvest time for fresh consumption, even though the amount of some phenolics and total phenolic substance are redused with progress of maturity.

ACKNOWLEDGEMENTS

The Research Project Coordination Unit of Suleyman Demirel University financially supported the presented study under the project number 4586-YL2-16.

REFERENCES

Alhendawi R.A., Römheld V., Kirkby E.A., Marschner H., 1997. Influence of increasing bicarbonate concentrations on plant growth, organic acid accumulation in roots and iron uptake by barley, sorghum and maize. Journal of Plant Nutrition, 20 (12): 1731-1753.

Al-Maiman S.A., Ahmad D., 2002. Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. Food Chemistry, 76: 437-441.

Ancos B., González E.M., Cano M.P., 2000. Ellagic acid, vitamin c, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. Journal of Agricultural and Food Chemistry, 48(10): 4565-4570.

Babou L., Hadidi L., Grosso C., Zaidi F., Valentao P., Valentao P.B., 2016. Study of phenolic composition and antioxidant activity of myrtle leaves and fruits as a function of maturation. European Food Research Technology, 242: 1447-1457.

Benzie I.F., 2003. Evolution of dietary antioxidants. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 136(1): 113-126.

Caponio F., Alloggio V., Gomes T., 1999. Phenolic compounds of virgin olive oil: influence of paste preparation techniques. Food Chemistry, 64: 203-209.

Choi S.H., Ahn J.B., Kim H.J., Im N.K., Kozukue N., Levin C.E., Friedman M., 2012. Changes in free amino acid, protein, and flavonoid content in jujube (*Ziziphus jujuba*) fruit during eight stages of growth and antioxidative and cancer cell inhibitory effects by extracts. Journal of Agriculture and Food Chemistry, 60: 10245-10255.

Doshi P., Adsule P., Banerjee K., 2006. Phenolic composition and antioxidant activity in grapevine parts and berries (*Vitis vinifera* L.) cv. Kishmish Chornyi (Sharad Seedless) during maturation. International Journal of Food Science and Technology, 41: 1-9.

Fadda A., Mulas M., 2010. Chemical changes during myrtle (*Myrtus communis* L.) fruit development and ripening. Scientia Horticulturae, 125(3): 477-485.

Gao Q.H., Wu C.S., Wang M., Xu B.N., Du L.J., 2012. Effect of drying of jujubes (*Ziziphus jujuba* Mill.) on the contents of sugars, organic acids, alpha-tocopherol, beta-carotene, and phenolic compounds. Journal of Agricultural and Food Chemistry, 60: 9642-9648.

Hochstein P., Atallah A.S., 1988. The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. Mutation Research/ Fundamental and Molecular Mechanisms of Mutagenesis, 202(2): 363-375.

Karaca E., 2011. The effect on phenolic compounds applied some of the procedures during production of pomegrane juice concentrate. Institute of Natural and Applied Science, University of Çukurova, MSc. Thesis, 144 pages, Adana, Turkey.

Karaçalı İ., 2010. Bahçe Ürünlerinin Muhafaza ve Pazarlanması (Storage and Marketing of Horticulture Products). Ege University, Faculty of Agriculture, Publication Number: 494 (Book in Turkish).

Kordis-Krapez K.M., Abram V., Kac M., Ferjancic S., 2001. Determination of organic acids in white wines by RP-HPLC. Food Technology and Biotechnology, 39(2): 93-99.

Kulkarni A.P., Aradhya S.M., 2005. Chemical changes and antioxidant activity in pomegranate arils during fruit development. Food Chemistry, 93: 319-324.

Lee W.J., Choi S.W., 2012. Quantitative changes of polyphenolic compounds in mulberry (*Morus alba* L.) leaves in relation to varieties, harvest period, and heat processing. Preventive Nutrition and Food Science, 17: 280-285

Mulas M., Fadda A., Angioni A., 2013. Effect of maturation and cold storage on the organic acid composition of myrtle fruits. Journal of the Science of Food and Agriculture, 93: 37-44

Ozgen M., Durgac C., Serce S., Kaya C., 2008. Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. Food Chemistry, 111: 703-706

Özhan-Tümer L., 2006. Changes on the amount of phenolic compound on maturation stages of some pomegranate varieties. Institute of Natural and Applied Science, University of Çukurova, MSc. Thesis, 53 pages, Adana, Turkey.

Özsayın S., 2012. Determination of nutritional status, some fruit quality parameters and antioxidant activity of pomegranate orchards (*Punica granatum* L.) in Antalya region. Institute of Natural and Applied Science, University of Akdeniz, MSc. Thesis, 134 pages, Antalya, Turkey.

Rossi M., Giussani E., Morelli R., Lo-Scalzo R., Nani R.C., Torreggiani D., 2003. Effect of fruit blanching on phenolics and radical scavenging activity of high bush blueberry juice. Food Research International, 36: 999-1005.

Siriamornpun S., Weerapreeyakul N., Barusrux S., 2015. Bioactive compounds and health implications are better for green jujube fruit than for ripe fruit. Journal of Functional Foods, 12: 246-255.

Wang R., Ding Y., Liu R., Xiang L., Du L., 2010. Pomegranate: constituents, bioactivities and pharmacokinetics. In: Chandra R. (Ed), Pomegranate. Fruit, Vegetable and Cereal Science and Biotechnology, 4 (Special Issue 2): 77-87.

Wu C.S., Gao Q.H., Guo X.D., Yu J.G., Wang M., 2012. Effect of ripening stage on physicochemical properties and antioxidant profiles of a promising table fruit pear-jujube (*Zizyphus jujuba* Mill.). Scientia Horticulturae, 148: 177-184.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE EFFECT OF CERTAIN CLIMATIC PARAMETERS ON THE APRICOT TREE

Cristina MOALE[1], Adrian ASĂNICĂ[2]

[1]Research Station for Fruit Growing Constanta, 25 Pepinierei Street, Valu lui Traian, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Romania

Corresponding author email: moalecristina@yahoo.com

Abstract

The pedo-climatic conditions in the South-Eastern part of Dobrogea are favourable to the culture of the apricot tree; this species that loves warm weather has always found good conditions for growing and yielding in the South-Eastern part of Romania, and especially in Dobrogea. In this period, 6 Romanian and foreign apricot tree cultivars were studied at RSFG Constanta: 'Harcot', 'Auraş', 'Goldrich', 'Dacia', 'Fortuna' and 'Hungarian C.M.B'. Branch samples were harvested from these 6 cultivars with different ripening periods three days after the frost and they were analysed. This paper presents the manner in which certain apricot tree cultivars reacted to frost in the winter of 2012, 2013 and 2014, as well as the effect of the hail on July 11th, 2014 on the apricot production. The greatest losses caused by the frost were registered in the winter of 2012 as far as the fructiferous buds are concerned: 94% at 'Fortuna', 83% at 'Auraş', 82% at 'Goldrich' and 'Dacia', 77% at 'Harcot' and 65% at 'Hungarian C.M.B.'. The losses caused by the hail on July 11th, 2014 affected the production of the 'Dacia' cultivar by 40% and of the 'Hungarian C.M.B.' by 30%. The climatic changes that have been registered throughout the past 10 years have negatively influenced the culture of the apricot tree and the effects have been classified according to the cultivar and its biology, as well as to the topographic placement of the allotments. The studies that have been carried out, together with the obtained results demonstrate the importance of choosing the cultivar assortment taking into account the favourability of the area, as well as the importance of installing anti-hail nets when setting up fruit-growing plantations.

Key words: *climate change, Prunus armeniaca* L.*, resistance to temperature variations.*

INTRODUCTION

The apricot tree is one of the most valuable fruit-growing species, due to the fact that is precocious, its production is quite significant and the fruits are very appreciated for their organoleptic qualities; are demanded by the market, both for fresh and processing, being capitalised at convenient prices.
The apricot tree has higher requirements concerning the heat, the temperature being the limitative factor in terms of extending the specie. The trees burst after a period of 7-10 days with temperatures above the biological threshold (6.5 °C), while flowering and fruit setting occur if the temperature is at least 10-12 °C (Hoza, 2000).
The studies which were carried out over a period of several years by researchers in the agro-meteorological field consider Dobrogea, as well as the entire south-eastern part of Romania, to belong to the most favourability area for the apricot tree culture (Roman, 1992).
Previous researches have revealed that the impact of climatic changes upon fruit-growing species can already be felt. For instance, by the end of the 90's, the flowering of the trees in Germany occurred several days earlier (Chmielewschi et al., 2004 and 2005). The vegetative season in Europe became longer by 10 days in the past 10 years (Chmielewschi and Rotzer, 2002). Due to the early flowering of the trees, in certain regions of Europe there was noticed an increase in the risk of damage caused by late frosts (Anconelli et al., 2004; Sunley et al., 2006; Legave and Clauzel, 2006; Legave et al., 2008; Chitu et al., 2004 and 2008) or by the disorders in the pollination and fruit setting processes (Zavalloni et al., 2006).
The Black Sea Coast is situated in the area with the largest average annual sums of day length on the country's territory, sums which

surpasses 2250-2300 hours (Păltineanu et al., 2000).

Action to adapt to climate changes through an appropriate management of structure, rotation and technology of fruit crops require knowledge of regional and local characteristics of present and future climate and of assessment the associated risks. In the latest 50 years, according to the studies of the National Meteorology Administration (Bojariu, et al., 2015), the monthly average of air temperature exclusively presents growth trends, statistically significant over the whole Romania, during spring and summer. There are also rising trends of air temperature in the winter, for the central and north-eastern part of the country, but the percentage of stations showing significant trends is lower (Birsan and Dumitrescu, 2014). A gradual downward trend was also noted in the intensity of cold stress generated by minimum air temperatures below -15°C in the winter months, from 26 units of the "cold" in the 1961 to 1970 decade, to a range between 12 and 21 of "cold" units in the last four decades (1971-2010).

Chitu et al. (2015) found that between 1985-2014, the highest growth rate of both from all the months had been recorded in November, the trend (+1.3 °C in ten years for maximum and 0.94 °C for minimum) being statistically insured. It was also noted that if in the west and center part of the country, winters were becoming milder (temperature increase with more than 1.5 °C per decade, even if the trend is not statistically assured). In the eastern and especially in the southern part of Romania in the last 30 years, lower temperatures by 0.1 to 0.8 °C were recorded.

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

Throughout the entire world, the research concerning the apricot tree has among its main objectives the relationship between the climatic conditions and the apricot tree culture. In our country, this relationship has been studied by numerous authors: Burloi (1957), Bordeianu et al. (1961), Cojean (1961), Mănescu et al. (1975), Topor (1987, 2002, 2009), Stancu et al. (1989), Roman et al. (1992) and so on. The results obtained by all these studies corresponded to a certain period of time and to a certain assortment of cultivars which represented the material. As concerns the new apricot tree cultivars, the obtained results are correlated with the evolution of the main climatic elements recorded in the past ten years.

This paper deals with the manner in which frost and hail influenced the fruit production of certain apricot tree cultivars from Dobrogea in the years 2012, 2013 and 2014.

That is why each area promotes a specific assortment of cultivars, although some of the latter might be common for all the areas (Topor, 1995).

MATERIALS AND METHODS

The biological material utilised consisted of 6 apricot tree cultivars and represented 3 ripening groups:

- extra early: 'Fortuna', 'Auraş';
- early: 'Harcot', 'Goldrich';
- medium: 'Dacia', 'Hungarian C.M.B.'

Upon comparing the ripening period of the fruit we can observe that the 'Fortuna' and 'Auraş' ripened in the period between the 16th and the 30th of June, the 'Harcot' and 'Goldrich' cultivars ripened in the period between the 20th of June and the 10th of July, whereas the period for the 'Dacia' and 'Hungarian C.M.B.' cultivars ripened in the period between the 7th and the 20th of July.

The studied cultivars are new and were created at RSFG Constanta: ‘Fortuna’ and ‘Auraş’, as well as the apricot tree cultivars introduced in the zonal and national assortment: ‘Harcot’, ‘Goldrich’, ‘Dacia’ and ‘Hungarian C.M.B.’

The trees were planted at a distance of 5 m between rows and 4 m between trees within the row (500 trees/ha).

The canopy shape is a Veronese vase and the trees were planted in 2003.

The applied culture technology is the one specific to the apricot tree: pruning, phytosanitary treatments, soil works, irrigation, harvesting, conditioning and capitalisation of the fruit.

Due to climatic changes over the past few years, the resistance of apricot trees seems to have become very different from one year to another. However, there are other factors involved as well, such as the topographic position of the orchard lot in which the apricot trees are planted (in the case of the studied cultivars the land was the same – a plateau), the alternation between minimum and maximum temperatures during winter, which renders the trees less resistant and last but not least, the severity of climatic accidents. The numbers of studied buds in the three years are shown in Figure 1.

The observations and determinations were carried out in the plots where there are some of the promoted apricot tree cultivars. Branch samples from the 6 cultivars were collected and analysed. The degree of differentiation of the flowering buds was relatively good. As concerns the soil where the plantation is placed, it is a calcareous chernozem (CZKa), with a loamy texture and a low alkaline pH (8.2) on its entire profile.

In addition, the overall climatic conditions were favourable to the growth and fructification of the trees, with exception of the years 2012-2014, when a very strong frost was registered in both January and February, leading to the loss of some of the floriferous buds, while the hail on July 11th, 2014 affected the production of the ‘Dacia’ and ‘Hungarian C.M.B.’ cultivars. With regard to these cultivars we observed the main fructification phenophases: the beginning of the blossoming, upon the appearance of the pink button; the beginning of the flowering, upon the appearance of the first open flowers; the ending of the flowering, when most of the flowers have lost their petals.

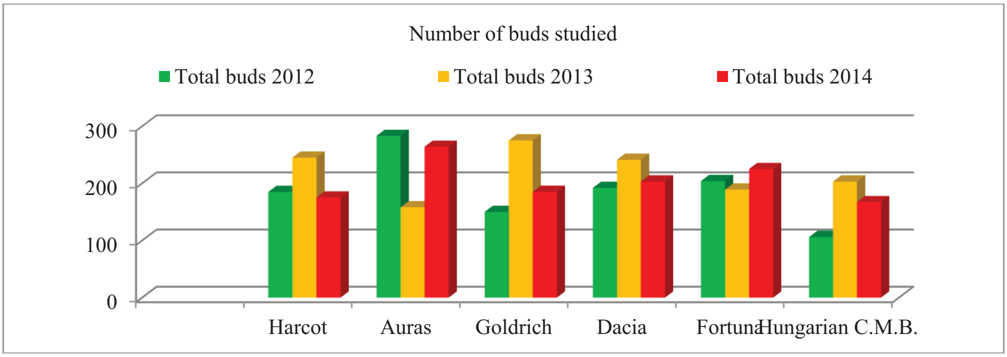


Figure 1. The number of studied buds per cultivar in the years 2012, 2013, 2014.

The duration of the flowering phenophase at a certain cultivar can vary according to the action of the maximum temperatures during the day and the intensity of the wind, correlated with the degree of differentiation of the trees (i.e. the amount of flowers per tree). The intensity of the flowering was graded on scale from 0 to 5, 0 being used when the cultivars displays no flowers at all, while 5 is used when the cultivar displays a plethora of flowers. The hardening of the core was determined by means of piercing it with a needle at regular intervals, usually 2 days. The process was carried out progressively, in the same day for all the observed cultivars. The harvesting maturity is largely influenced by a series of climatic and agro-technical factors, such as: temperature, drought, quantity of fruit per tree, shape of the head, density of the trees etc. The observations and determinations were carried out 3-5 days after the climatic accidents recorded in 2012, 2013 and 2014, respectively and the production was assessed after the hail occurrence on July 11^{th}, 2014. The hail, with a dimension of approximately 5-20 mm, seriously damaged the fruit production of some of the apricot tree cultivars, more exactly those who had not been harvested until July 11^{th}, 2014. The climatic data were recorded with the aid of an automatic meteorological station (the WatchDog type) and were processed as daily averages. We noticed that the resistance of apricot tree cultivars differs from one year to the next because of the climatic changes that have occurred during the past few years and it depends on the gravity of climatic accidents. The minimum and maximum temperatures during winter alternate and together with the gravity of climatic accidents lead to the weakening of the trees.

The behaviour of apricot tree cultivars towards the attack of the pathogen agents - 1) *Stigmina carpophila* (Lév.) M.B. Ellis, 2) *Cytospora cincta* Sacc andi 3) *Monilinia laxa* (Aderhol et Ruhl.) - was studied under conditions of natural infections, according to the test created by Crossa Raynaud (1968).

The evaluation technique consisted in writing down the frequency of the attacked organs and the intensity with which the symptoms manifested themselves and these two aspects were utilised in assessing the behaviour of the cultivars.

The field observations were centred on the calculation of the pathogens' frequency (F %) and intensity (I) on different tree organs such as: leaves, flowers, shoots, branches and fruits. For the intensity of the diseases marks were granted on a scale from 0 to 4.

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

F.A.= cultivars without attack (F%= 0 and I= 0)
T= tolerant cultivars (F%= 0.1-5% and I= 0 $\underline{\scriptstyle\pm}$ +)
S.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=+ $\underline{\scriptstyle 2}$ 4)
F.S.= highly sensitive cultivars (F%= 50.1% - 100%, I= + $\underline{\scriptstyle 4}$ 4)

Table 1. Cultivar categorisation into classes and groups of resistance

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

RESULTS AND DISCUSSION

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

In the period 2012-2014 the blossoming of the floriferous buds of the apricot trees occurred between the following limits: between 08.03 and 14.03 for the 'Fortuna' cultivar, between 11.03 and 27.03 at the 'Auraș' cultivar, between 15.03 and 27.03 at the 'Harcot' cultivar, between 24.03 and 29.03 at the 'Goldrich' cultivar, between 13.03 and 28.03 at the 'Dacia' cultivar, between 24.03 and 29.03 at the 'Hungarian C.M.B.' cultivar. Calendaristically the blossoming at the apricot tree occurred between 08.03 and 29.03 (21 days) in the studied years 2012-2014 (Table 2).

Table 2. The main stagers of fructification and apricot in the 2012-2014 period

No.	Cultivar	Year	The swelling of the flowering buds	The flowering			Inten-sity	The hardening of the stone	Harvesting maturity
				Beginning	Ending	Duration (days)			
1	Fortuna	2012	08.03	18.03	05.04	17	1	04.06	14.06
		2013	14.03	16.03	01.04	15	1	10.06	27.06
		2014	12.03	21.03	02.04	11	1	07.06	25.06
		Limits	08.03-14.03	16.03-21.03	01.04-06.04	11-17	1	04.06-10.06	14.06-25.06
2	Auraș	2012	11.03	22.03	06.04	14	2	04.06	12.06
		2013	27.03	28.03	10.04	12	3	08.06	17.06
		2014	22.03	17.03	03.04	16	4	10.06	29.06
		Limits	11.03-27.03	17.03-28.03	03.04-10.04	12-16	2-4	04.06-10.06	12.06-29.06
3	Harcot	2012	15.03	25.03	10.04	15	2	02.06	18.07
		2013	29.03	28.03	16.04	18	2	08.06	16.07
		2014	26.03	20.03	03.04	13	4	08.06	27.07
		Limits	15.03-27.03	20.03-28.03	03.04-16.04	13-18	2-4	02.06-08.06	16.07-27.07
4	Goldrich	2012	26.03	04.04	17.04	13	2	06.06	13.07
		2013	29.03	09.04	23.04	14	2	10.06	18.07
		2014	24.03	20.04	28.04	8	2	08.06	16.07
		Limits	24.03-29.03	04.04-20.04	10.04-28.04	8-14	2	06.06-10.06	13.07-18.07
5	Dacia	2012	22.03	05.04	20.04	15	3	08.06	03.07
		2013	28.03	11.04	19.04	8	3	10.06	02.07
		2014	13.03	20.04	30.04	10	4	07.06	12.07
		Limits	13.03-28.03	05.04-20.04	19.04-30.04	8-15	2-4	07.06-10.06	02.07-12.07
6	Hungarian C.M.B.	2012	24.03	05.04	18.04	13	4	07.06	17.07
		2013	29.03	09.04	16.04	7	3	09.06	15.07
		2014	25.03	18.04	27.04	9	4	10.06	19.07
		Limits	24.03-29.03	05.04-18.04	16.04-27.04	7-13	3-4	07.06-10.06	15.07-19.07

The beginning of the flowering. For all the studied cultivars the beginning of the flowering in the period 2012-2014 was recorded; however, the cultivars entered this phenophases at different times, albeit not necessarily significant (a few days from one cultivar to the next), so that mutual pollination was fully ensured. The limits for this phenophase were 16.03 and 20.04.

The ending of the flowering. In the studied period 2012-2014 the ending of the flowering occurred between 01.04 and 06.04 for the 'Fortuna' cultivar, between 03.04 and 10.04 for the 'Auraș' cultivar, between 03.04 and 16.04 for the 'Harcot' cultivar, between 10.04 and 28.04 for the 'Goldrich' cultivar, between 19.04 and 30.04 for the 'Dacia' cultivar, between 16.04 and 27.04 for the 'Hungarian C.M.B.' cultivar. The dates were recorded as the days when the flowers lost their last petals. The duration of the flowering at the peach tree (average for the three studied years) expressed in number of days varied between 7 days (the 'Hungarian C.M.B.' cultivar in 2013) and 18 days (the 'Harcot' cultivar in 2013).

The intensity of the flowering. In 2012, 2013 and 2014 the following cultivars displayed a weak intensity of the flowering: 'Fortuna' - 1 (2012, 2013 and 2014), 'Auraș' - 2 (2012), 'Harcot' - 2 (2012, 2013) and 'Goldrich' - 2 (2012, 2013 and 2014).

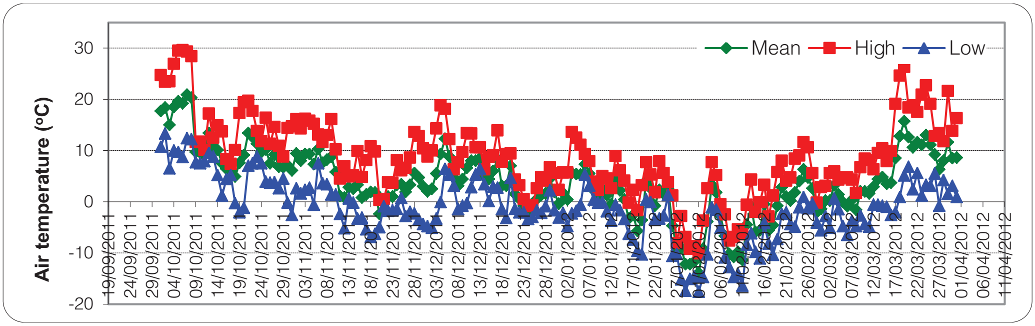
The hardening of the core. This phenophase occurred in the first half of the month of June (between 2^{th} and 10^{th}) in the years 2012, 2013 and 2014.

The harvesting maturity. Each ripening period has large variation limits from one year to another, depending on how the climatic factors determine the type of vegetation in a specific year: early, late or extra late. The harvesting maturity of the fruit had as variation limits the 14th of June and the 27th of July.

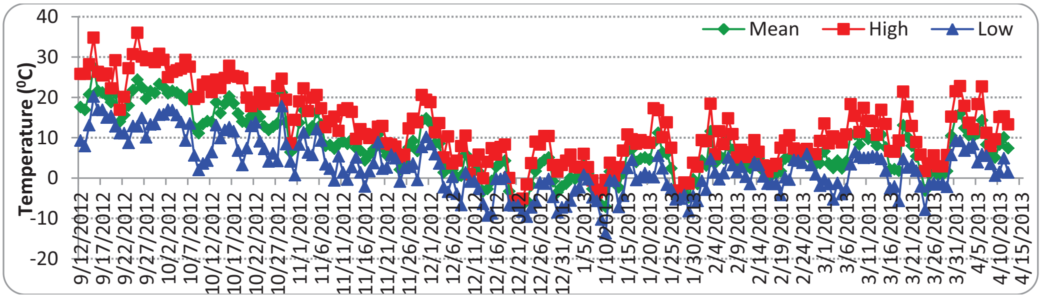
As we can notice in figure 2a, January 2012 was the coldest month, during which 9 days recorded daily average temperatures ranging from -10.2 °C and -17.6 °C. These values, together with those that were extremely varied in February (7 days with daily average temperatures of -10.4 and -16.4 °C) and eight consecutive days of hoarfrost, the ice on the branches caused the loss of 65% - 94% of the floriferous buds at the studied cultivars**.**

Figure 2b. reveals the fact that the coldest month in the period September 2012 - April 2013 was January 2013, when the recorded values were -13.7 °C (January 10th, 2013). These values did not significantly influence the loss of floriferous buds at the apricot tree cultivars (local observations).

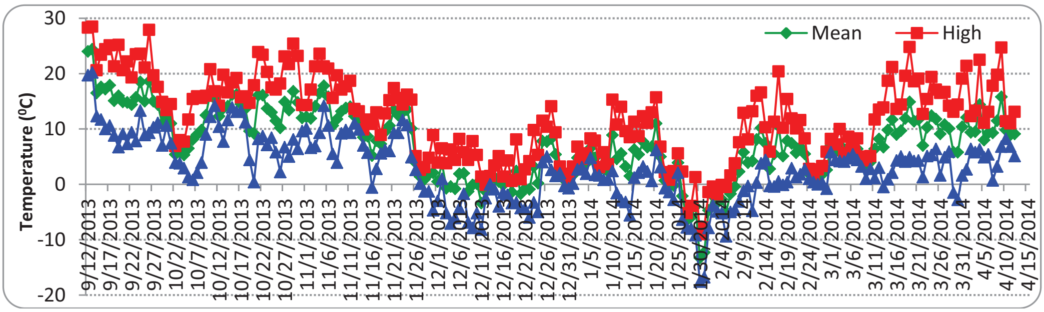
In the period October 2013 - March 2014 (Figure 2c.) the lowest temperature was recorded in January: -17.6 °C (January 30th, 2014); another day when the recorded temperature was low (-9.4 °C) was February 5th, 2014. The low temperatures recorded during this period affected the 'Goldrich' cultivar (67%) and the 'Fortuna' cultivar (90%).



2 a



2b



2c

Figure 2. Air temperature (°C) in the cold period October 2011 – March 2012 (a), October 2012 – March 2013 (b) and October 2013 – March 2014 (c) at Valu lui Traian, Constanţa

The observations were carried out with the aim of assessing the losses of floriferous buds because of temperature variations during winter and the low temperatures during the day.
Thus, for the ‘Harcot’ cultivar the losses recorded for 2012 were of approximately 77%, 58% for 2013 and 42% for 2014, there being difference from one cultivar to another. The winter frost caused losses for the ‘Goldrich’ cultivar of 82% in 2012, 69% in 2013 and 67% in 2014. For the ‘Dacia’ cultivar, the losses were of 82% in 2012, 63% in 2013 and 28% in 2014. The ‘Auraş’ cultivar recorded losses of 83% in 2012, 65% in 2013 and 24% in 2014. For the ‘Fortuna’ cultivar, the losses were of 94% in 2012, 92% in 2013 and 90% in 2014. The ‘Hungarian C.M.B.’ cultivar recorded losses of 65% in 2012, 54% in 2013 and 15% in 2014 (Figure 3).

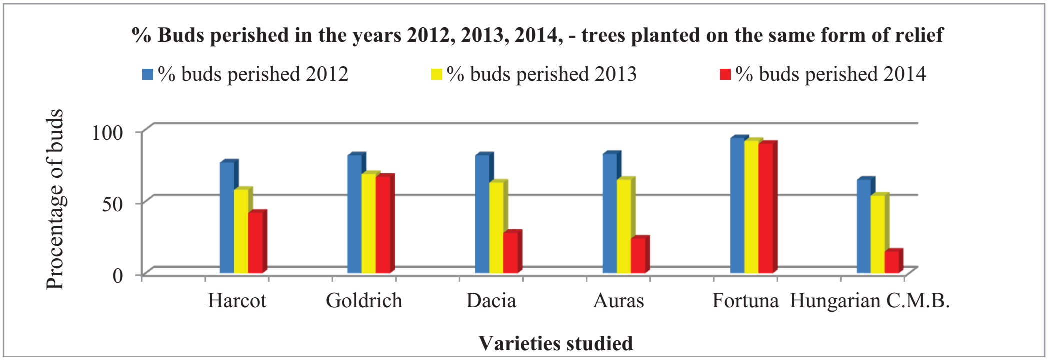


Figure 3. Procentage of apricot tree flowering buds perished due to frosts during the winter of 2012, 2013 and 2014 at Valu lui Traian, Constanta

We must bear in mind the fact that the losses caused by the winter frost of 2012, together with those caused by hoarfrosts and late frosts were very severe, taking also into account the surface of the Station’s orchards cultivated with this cultivar. These losses were also caused by the warm period before the frost – in the first three weeks of January 2012 the average temperature of the air was positive, of approximately 5 °C.
A good resistance to frost during the winter of the three studied years was displayed by the apricot cultivar, with the following percentages: ‘Hungarian C.M.B.’- 45%, (Figure 4).

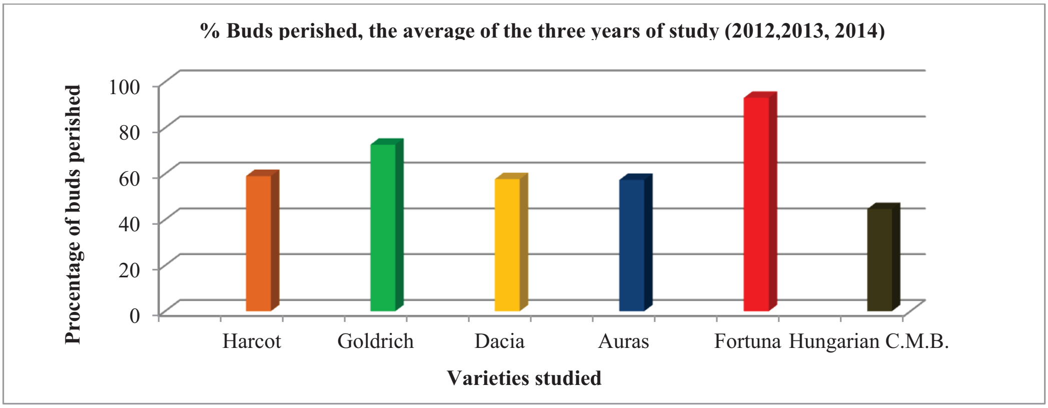


Figure 4. Procentage of apricot tree flowering buds perished because of frosts (average over the three years), Valu lui Traian

In these conditions, the ‘Auraş’, ‘Dacia’ and ‘Harcot’ cultivars were more than 50% damaged. The climatic accidents recorded in January and February 2012 (sudden temperatures of -16.4 °C, minimum temperature during the day) and 8 days of hoarfrost caused the damaging of the production for the early cultivars ‘Fortuna’ and ‘Goldrich’.

At R.S.F.G. Constanta, in the second week of June 2014, more exactly on July 11th, the amount of precipitations was accompanied for 10 minutes by hail, which affected 40% of the fruit production for the ‘Dacia’ cultivar (the fruit were just beginning to ripe) and 30% for the ‘Hungarian C.M.B.’ cultivar (Figure 5). The hail bruised the fruit, the shoots and the stems, thus creating a good environment for future infections and diseases. The bruises on the fruit, despite some of them becoming scars, diminished the commercial aspect and the quality of the production.

Figure 5. The ‘Dacia’ cultivar affected by the hail on July 11th, 2014 (full maturity)

Table 3, presents the relative sensitivity of 6 cultivars from the demonstrative plot created within the laboratory responsible with improving the apricot tree concerning the attack of the pathogens *Stigmina carpophila*, *Cytospora cincta* and *Monilinia laxa*, under natural conditions of infection. The analysis of the data in this table highlights a variation in the apricot tree cultivars’ behaviour towards a pathogen or another.

Table 3. The behaviour of apricot tree cultivars towards the attack of the main pathogens in the period 2012-2014

No.	Cultivar	Year	Intensity of attack (note)			
			Stigmina carpophila	*Cytospora cincta*	*Monilinia laxa*	*Monilinia fructigena*
a. Cultivars planted in 2003						
1.	Harcot	2012	FA	T	T	T
		2013	FA	T	T	T
		2014	FA	T	T	T
2.	Auraș	2012	T	FA	FA	FA
		2013	T	FA	FA	FA
		2014	T	FA	FA	FA
3.	Goldrich	2012	FA	S	T	T
		2013	FA	SA	T	T
		2014	FA	SA	T	T
4.	Dacia	2012	FA	S	SA	SA
		2013	FA	SA	SA	SA
		2014	FA	SA	S	S
5.	Fortuna	2012	SA	FA	FA	FA
		2013	SA	FA	FA	FA
		2014	SA	FA	FA	FA
6.	Hungarian C.M.B.	2012	FA	T	SA	SA
		2013	FA	T	SA	SA
		2014	FA	T	S	S

According to the intensity (I) of the attack the studied cultivars were classified as follows (Table 3): *Stigmina carpophila* in 6 resistance classes: **cultivars without attack (F.A.)** – 4 cultivars belong to this class, where the intensity (I) of the attack was 0: ‘Harcot’, ‘Goldrich’, ‘Dacia’ and ‘Hungarian C.M.B.’, during all three studied years (2012, 2013 and 2014). The ‘Auraș’ cultivar was not attacked by *Stigmina carpophila*; **tolerant cultivars (T)** – ‘Auraș’ in all three studied years; **weakly**

attacked (S.A) - ‘Fortuna’ (in 2012, 2013 and 2014.

The *Stigmina carpophila* fungus survives from one year to the next in buds, in cracks in the bark and in wounds, which facilitates the occurrence of infections in early spring, at temperatures above 8 °C and an atmospheric humidity of over 80%. Temperatures over 30°C stop the evolution of the disease.

By correlating the frequency of the attack (F%) of *Cytospora cincta* Sacc with its intensity (I), the studied cultivars were categorised as follows: **cultivars without attack (F.A.) -** both the frequency (F%) and the Intensity (I) were 0; there are 2 cultivars in this class: ‘Auraș’, and ‘Fortuna’, in all three studied years; **tolerant cultivars (T)** – ‘Harcot’ and ‘Hungarian C.M.B.’. in the studied years 2012, 2013 and 2014; **weakly attacked (S.A)** – ‘Goldrich’ and ‘Dacia’ were weakly attacked in 2013 and 2014; **sensitive (S)** – ‘Goldrich’ and ‘Dacia’. None of the studied cultivars could be categorised into the classes **moderately resistant (M.A.)** and **highly sensitive (F.S.).**

The disease caused by the *Cytospora cincta* Sacc fungus manifested itself through the sudden drying of the branches. The affected trees seemed to be suffering in full summer the leaves wilted, becoming yellowish-green and the drying of the branches occurred from the tip towards the base.

As far as the attack of the *Monilinia laxa* and *Monilinia fructigena* fungi is concerned (Aderh et Ruhl) Honey, the disease manifested itself on all aerial organs during spring, through the wilting of the flowers and the drying of the vegetative buds and shoots.

The strong attack of the *Monilinia laxa* fungus occurs when the maximum phase of pathogenicity or the fungus is corroborated with the maximum phase of receptivity of the trees (the flowering phase), directly conditioned by temperature and humidity. This explains why the intensity of the attack on the same cultivar varies from one year to another, remaining, though, within the limits of the minimal, medium or maximal attack levels. Certain factors favour the attack of the *Monilinia*, among which: the biological reserve of the pathogen from one year to the next, given the fact that the fungus winte rs as a mycelium within the branches, at the base of the flowering buds, or in mummified fruit that are still on the branches; the atmospheric humidity and the air temperature during the flowering; the genetic resistance of the cultivars.

By correlating the frequency of the attack (F %) with its intensity (I), the studied cultivars were categorised as follows: **cultivars without attack (F.A.)** – 2 cultivars, for which both the frequency (F%) and the intensity (I) were 0: ‘Auraș’ and ‘Fortuna’, in all three studied years; **tolerant cultivars (T)** - 2 cultivars: ‘Harcot’ and ‘Goldrich’, in 2012, 2013 and 2014; **weakly attacked (S.A)**: ‘Dacia’ and ‘Hungarian C.M.B.’ in 2012 and 2013; **sensitive (S)** – ‘Dacia’ and ‘Hungarian C.M.B.’ in 2014. None of the studied cultivars could be categorised into the classes **moderately resistant (M.A.)** and **highly sensitive (F.S.)**.

Most studied cultivars manifested an increased resistance towards the attack of the pathogen agent *Monilinia laxa*, with the exception of ‘Dacia’ and ‘Hungarian C.M.B.’ cultivars, which were sensitive in 2014. These cultivars were affected by the hail that occurred on July 11th, 2014, which facilitated the development of moniliasis especially at cultivars that were in full harvesting maturity.

CONCLUSIONS

The greatest yield losses were recorded for the ‘Fortuna’ cultivar in 2012 – 94% in 2013 - 92% and 90% in 2014.

The lower losses during the three studied years were recorded by ‘Hungarian C.M.B’.

The hail from July 11th, 2014, which lasted for only 10 minutes, affected the ‘Dacia’ cultivar (40%) and the ‘Hungarian C.M.B.’ cultivar (30%) and therefore we recommend the orchards to be covered with anti-hail nets.

As far as the attack of the *Stigmina carpophila* (Lév.) M.B. Ellis fungus is concerned, the following cultivars: ‘Harcot’, ‘Goldrich’, ‘Dacia’ and ‘Hungarian C.M.B.’ – proved to be tolerant in all three studied years (2013-2015).

Concerning the attacks of the *Cytospora cincta* fungus, both the sensitivity and the resistance towards the pathogen are exclusively connected to the soil.

The sensitive cultivars were 'Goldrich' and 'Dacia' in 2012, while the resistant ones, without attack were 'Auraș' and 'Fortuna' (2013, 2014, 2015).

Most studied cultivars manifested an increased resistance towards the attack of the pathogen agent *Monilinia laxa*, with the exception of 'Dacia' and 'Hungarian C.M.B.' cultivars, which were sensitive in 2014. These cultivars were affected by the hail that occurred on July 11th, 2014, which facilitated the development of moniliasis especially at cultivars that were in full harvesting maturity.

REFERENCES

Anconelli S. Antolini G. Facini O. Giorgiadis T.Merletto V. Nardino M. Palara U. Pasquali A.Pratizzoli W. Reggitori G.Rossi F. Sellini A. Linoni F, 2004: Previsione e difesa dalle gelate tardive – Risultati finali del progetto DISGELO. CRPV Diegaro di Cesena (FO). Natiziario tecnico N.70. ISSN 1125-7342. 64. pp.

Bordeanu, T., Tranavski, I., Radu, I.F. 1961. Study on Winter Rest and Biological Threshold of Apricot Flower Buds. Studies and research of Biology. Plant Biology Series no. 4 (XIII).

Burloi Niculina. 1957. Behavior apricot buds in the Bucharest. Communications of the Romanian Academy no. 9, vol. VII, Bucharest

Birsan, M.V., Dumitrescu, A., 2014. ROCADA: Romanian daily gridded climatic dataset (1961-2013) V1.0. National Meteorological Administration, Bucharest Romania, doi: 10.1594/PANGAEA.833627.

Bojariu, R., Bîrsan, V.M., Cică, R., Velea, L., Burcea, S., Dumitrescu, A., Dascălu, S.I., Gothard, M., Dobrinescu, A., Cărbunaru, F., Marin, L., 2015. Climatical changes - from physical bases to risks and adaptation. Printech Publishing House, Bucharest, ISBN 978-606-23-0363-1, 200 pp.

Chitu E., M. Butac, S. Ancu and V.Chitu 2004. Effects of low temperatures in 2004 on the buds viability of some fruit species grown in Maracineni area. Annals of the University of Craiova. Vol. IX (XLV), ISSN 1435-1275: 115-122.

Chițu E., D. Sumedrea, Cr. Pătineanu, 2008. Phenological and climatic simulation of late frost damage in plum orchard under the conditions of climate changes foreseen for România. Acta Horticulturae (ISHS) 803:139-146.

Chitu, E., Giosanu D., Mateescu E., 2015. The Variability of Seasonal and Annual Extreme Temperature Trends of the Latest Three Decades in Romania. Agriculture and Agricultural Science Procedia. Volume 6, 2015, doi:10.1016/j.aaspro.2015.08.113, pages 429-437.

Chițu, E., Elena Mateescu, Andreea Petcu, Ioan Surdu, Dorin Sumedrea, Tănăsescu Nicolae, Cristian Păltineanu, Viorica Chițu, Paulina Mladin, Mihail Coman, Mădălina Butac, Victor Gubandru, 2010. Methods of estimating climatic favorability for tree culture in Romania. The Publishing House INVEL Multimedia, CNCSIS accredited, ISBN 978-973-1886-52-7.

Chmielewski F.M., Muller A., Kuchler W., 2005. Possible impacts of climate change on natural vegetation in Saxony (Germany). Int. J. Biometeorol, 50:96-104.

Chmielewski F.M., Rotzer T 2002. Annual and spatial variability of the begenning of growing season in Europe in relation to air temperature changes. Clim. Res. 19(1), 257-264.

Chmielewski F.M., Muller A., Bruns E., 2004. Climate changes and trends in phenology of fruit trees and field crop in Germany, 1961-2000, Agricultural and Forest Meteorology 121 (1-2), 69-78.

Cojean Natalia. 1961. Rezistenţa la îngheţ a mugurilor floriferi de cais în legătură cu etapele de creştere şi de dezvoltare. Studii şi cercetări de Biologie. Seria Biologie vegetală nr.1.

Crossa-Raynaud, P.H. (1969). Evaluating Rezistance to *Monilinia laxa* (Aderh&Ruhl) Honey of varieties and hybrids of apricots and almonds using mean growth rate of cankers on young branches as criterion of susceptibility. J. Amer. Soc. Hort. Sci. 94, 282-284.

Hoza D., 2000. Pomologie. Ed.Prahova, Ploiești, pp: 286, ISBN 973-99268-3-5.

Legave, J.M. and Clazel G., 2006. Long-term evolution of flowering time in apricot cultivars grown in southern France: wich future imtacts of global warming? Acta Horticulturae, 714: 47-50.

Legave, J.M., Farrera, I., Almeras, T. and Calleja, M, 2008. Selecting models of apple flowering time and understading how global warming has had an impact on this trait. Journal of Horticultural Science & Biotechnology, 83:76-84.

Olensen, J.O., Bindi, M., 2002. Consequences of climate change for European agricultural productivity, land use and policy. European Journal of Agronomy, 16, 239–262.

Mănescu Creola, Baciu Elena And Cosmin Silvia, 1975. Controlul biologic în pomicultură. Ed. Ceres, Bucureşti.

Paltineanu, Cr., Mihailescu, I.F. & Seceleanu, I. (2000A). Dobrogea, condiţiile pedoclimatice, consumul si necesarul apei de irigatie ale principalele culturi agricole. Editura Ex Ponto, Constanta, 258pp.

Roman Ana Maria, Cusursuz Beatrice, Cociu V., Elena Topor, S.A. 1992. Studiul agrometeorologic al mecanismelor exogene ce controlează înflorirea unor specii prunoidee din Romania. Eucarpia, Angers, Franta.

Stancu, T., Balan Viorica, Ivascu Antonia, Cociu, V.,1989. Resistance to frost and wintering of some apricots varieties with different geographical origin, under Romanian plain conditions. Acta Horticulturae, nr. 239.

Sunley, R.J., Atkinson, C.J. and Jones, H.G., 2006. Chill unit models and recent changes in the occurrence of winter chill and soring frost in the United Kingdom. Jurnal of Horticulturae. Science & Biotechnology, 81: 949-958.

Sumedrea D., Tănăsescu N., Chițu E., Moiceanu D., Marin Fl., Cr., 2009. Present and perspectives in Romanian fruit growing technologies under actual global climatic changes. Scientific Papers of the Research Institute for Fruit Growing Pitesti, Vol. XXV, ISSN 1584-2231, Editura INVEL Multimedia, București: 51-86.

Topor Elena, 1995. Cercetări privind îmbunătățirea sortimentului de cais în Dobrogea. Teză de doctorat ASAS București.

Topor Elena, 1987. Modificările continutului in hidrati de carbon în funcţie de perioada de vegetaţie a 11 soiuri de cais. Vol. Omagial, S.C.D.P. Constanta.

Topor Elena, 2002. Cercetari privind relaţia dintre condiţiile climatice si fructificarea caisului. Lucr. St. Vol.1 . Seria Horticultura, Ed. Ion Ionescu de la Brad Iasi.

Topor Elena, 2009. Cercetari privind influenţa schimbarilor climatice asupra culturii caisului în Dobrogea. Lucrările Simpozionului Mediul si Agricultura în regiunile aride –prima editie. ISBN 978-973-7681-68-3.(223)

Zavalloni, C., Andersen, J.A., Flore, J.A., Black, J.R. and Beedy, T.L., 2006. The pileus project: climate impacts on sour cherry production in the great lakes region in past and projected future time frames. Acta Horticulturae, 707: 101-108.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EXAMINATION OF THE POMOLOGICAL CHARACTERISTICS AND THE PRESENCE OF HEAVY METALS IN THE PEACH CULTIVAR ‘‘CRESTHAVEN’’ FROM REPUBLIC OF MACEDONIA

Viktorija STAMATOVSKA[1], Ljubica KARAKASOVA[2], Gjore NAKOV[3], Tatjana KALEVSKA[1], Marija MENKINOSKA[1], Tatjana BLAZEVSKA[1]

[1]"Saint Clement of Ohrid" University of Bitola, Faculty of Technology and Technical Sciences, Dimitar Vlahov bb, 1400 Veles, Republic of Macedonia
[2]"Ss. Cyril and Methodius" University, Faculty for Agricultural Sciences and Food, Aleksandar Makedonski bb Blvd., 1000 Skopje, Republic of Macedonia
[3]"Angel Kanchev" University of Ruse, Department of Biotechnology and Food Technologies, Branch Razgrad, 47 Aprilsko vastanie Blvd., Razgrad 7200, Bulgaria

Corresponding author email: vikistam2@gmail.com

Abstract

The pomological characteristics are very important for each type of fruit. The different types of fruits are being classified according to their shape, size, dimensions and the other features. The purpose of this paper is to examine the pomological characteristics of the peach fruits from ‘‘Cresthaven’’ cultivar, from Rosoman, Republic of Macedonia. The fruits are being collected in full technological maturity. The following characteristics have been determined: height, width and thickness of the fruit (using a caliper with an accuracy of 0.01 mm), weight of the fruit and weight of the mesocarp and pit (using an analytical balance with an accuracy of 0.001 g), and the yield is also mathematically calculated. Also, during the research have been conducted an analysis for the presence of heavy metals (Pb, As and Cd) in the fruits (using atomic absorption spectrometer). The research was repeated three times, in a period of three years (2011, 2012, 2013). In the three-year period of examinations, from a statistical point of view, between the calculated values of the examined parameters of the peach fruits there are no statistically significant differences, with the exception of the pit weight and the yield, for which statistically significant differences have been determined. The contents of Pb, As and Cd in the fruits in three-year testing period are in accordance with the applicable rules and prescribed norms for food safety in the Republic of Macedonia.

***Key words**: peach, pomological characteristics, heavy metals.*

INTRODUCTION

The peach is one of the most widespread fruits in the world because of its specific pleasant taste, juiciness and nutritional values. The peach, along with the cherries, sour cherries, plums and apricots, belongs to the group of stone fruits (Kantoci, 2008; Jašić, 2007). Peach originates from China, and today is being cultivated in warm temperate regions of Europe, Asia, North America, parts of Africa and Australia. Peach fruits can be consumed fresh and processed in different ways (Mratinić, 2000).

According to the systematic classification, the peach fruit belongs to the family *Rosaceae*, subfamily *Prunoidea*e, genus *Prunus*, with a greater number of species (Bulatović-Danilović, 2007). Known varieties: ‘Springtime’, ‘Suncrest’, ‘Springold’, ‘Sprincrest’, ‘Redtop’, ‘Redhaven’, ‘Cresthaven’ and other (Kantoci, 2008).

The fruit of the peach is big, usually with a round shape, juicy, with a pleasant sweet-sour taste and specific aroma. From the total weight of the fruit, which ranges from 80 to 250 g (sometimes more), the part that can be consumed amounts 93 to 98% (Mratinić, 2000). It is known that the peach fruit contains carbohydrates, organic acids, pigments, phenolic compounds, antioxidants and traces of proteins and lipids. It is a rich source of potassium, iron, fiber, vitamin A, vitamin C and other vitamins (Crisosto and Valero, 2008; Hajilou et al., 2013).

The natural conditions for the cultivation of peaches in Macedonia are very favorable. With the construction of more factories for fruit processing, peach became much demanded raw material. The increased interest in this fruit affected the increasing number of peach trees. Thus, from 327.000 in 1970 their number increased in 2010 up to 505.000, and the output was 10.200 tons. The cultivation of peaches is most widespread in the Tikves region, then in the regions of Skopje, Strumica, Radovis, Gevgelija and Kumanovo (Stojmilov and Apostolovska-Toševska, 2016). Different varieties have been cultivated, and one of the most known is the 'Cresthaven' peach cultivar from the region of Rosoman.

The features of the fruits of peach cultivar 'Cresthaven' have been presented by many authors (Mratinić, 2000; Bulatović-Danilović, 2007; Nenadović-Mratinić et al., 2003). This variety has been obtained by complex hybridization. Peach ripens from mid to late August. The fruit is round in shape, firm and belongs to the group of large fruits from 250 g. It features with an average weight of 184 g, length of 6.75 cm, width of 7.41 cm and thickness of 7.56 cm. The main color of the exocarp which is yellow is complemented with red color, which covers most of the surface of the fruit (50-60%). The mesocrap is yellow, juicy, firm, tasty and of high quality. The pit can be easily removed from the fruit's flash. Peaches can handle transportation. Fruit fresh include: 13.12% soluble dry matter, total sugars 10.43% and 0.60% total acids.

The quality of the fruits expressed by their size, appearance and taste, is very important prerequisite for their sale. A review of the fruits should include mechanical, sensorial, chemical and microbiological control, as well as remarks for the variety.

The first remark refers to the type and variety, degree of maturity, the average mass, the fruit size, the percentage of seed in the fruit, the stalk, the petals, husk, etc. Pomological characteristics of the agricultural products are parameters for determining the appropriate standards for assessment, transportation, processing and packaging (Karakašova and Babanovska-Milenkovska, 2012).

According to Crisosto et al. (2004) cited by Milošević and Milošević (2011), the size is a quantitative inherited factor for determination of the yield of fruit, the quality and the acceptability of the consumers.

The fruit is part of the daily diet, so it is very important to know the possible presence of heavy metals in it. The data indicate that heavy metals are pollutants of the fruit and such fruit consumed by humans may pose health risks (Sobukola et al., 2010; Elbagermi et al., 2012; Matei et al., 2013; Chandorkar and Deota, 2013). Lead, cadmium and arsenic are among the most widespread toxic elements present in the food and the environment.They have a long half-life after absorption in humans and animals, which can cause unpleasant effects such as damage of internal organs, nervous system, kidneys, liver and lungs (Ghazanfarirad et al., 2014).

Considering the previously mentioned, we consider that the data obtained in this examination will be of interest for the manufacturers, as well for the consumers, too. The obtained data will define the pomological characteristics of the peach fruits of the cultivar 'Cresthaven', available in the market, and at the same time will bring awareness for the presence of heavy metals in them, which is very important from a health point of view.

MATERIALS AND METHODS

The peach fruits of the cultivar 'Cresthaven', cultivated in Rosoman, Republic of Macedonia, have been used as a testing material. The fruits are collected in full technological maturity. For analysis have been taken 50 healthy fruits without major and average weight of the fruit, mesocarp and pit, using analytical balance with an accuracy of 0,001 g, average fruit sizes (height, width and thickness), using a caliper with an accuracy of 0,01 mm, have been determined. The fruits have been examined in the Laboratory for fruit and vegetables processing at the Faculty of Agricultural Sciences and Food in Skopje, Republic of Macedonia. The yield is mathematically calculated. The mass ratio between the useful part and the part that is not used, expressed as a percentage represents the yield (Karakašova, 2011). The contents of As, Pb and Cd have been determined at the Institute of Food at the Faculty of Veterinary Medicine - Skopje using atomic absorption spectrometry according SOP 392. The research was repeated three times, in a period of three years.

The results of the examination are presented, analyzed and statistically processed using the computer program Microsoft Excel and the statistical package SPSS Statistics Version 19.

RESULTS AND DISCUSSIONS

The results from the research of the pomological characteristics of fruits of peach cultivar ‘Cresthaven’ are presented in tables (Table 1) and graphics (Figure 2).
The results represent mean values obtained from analysis of the fruits used in each production year (2011, 2012, 2013).
Analyzed fruits of peach variety of ‘Cresthaven’ (Figure 1) are characterized with features inherent for the type and variety (Bulatović-Danilović, 2007).
The fruit is big, solid and round shape. The surface is yellow, complemented by red color. The mesocarp is yellow with redness around the pit, which can be easily split (Vuletić, 2016). The smell is pleasant and the taste is sweet.

Figure 1. Peach cultivar ‘Cresthaven’

In Table 1 are shown the obtained mean values for mass and dimensions for the peach fruits from the variety ‘Cresthaven’, and the obtained mean values for the mass of the mesocrap and of the pit and the yield calculated.
The mass of the fruit is one of the major pomological features, which largely affects the yield (Nikolić et al., 2013). Based on the obtained values for the average mass of the fruits in the three year period (from 169.08 ± 26.99 to 174.24 ± 44.39 g), it can be concluded that this variety is characterized with large fruits (150-200 g) (according to Mratinić, 2012 quoted Vuletić, 2016). The high standard deviation value of mass clears good selection of samples.

Table 1. Pomological characteristics of fruits of peach cultivar ‘Cresthaven’ in 2011, 2012, 2013

Year	Analyzed parameters	n	$\bar{x}$	SD	*p- value*		
					2011	2012	2013
2011	Mass of the fruit (g)	50	174.24	44.39		0.586	0.489
	Height of the fruit (cm)	50	6.30	0.55		0.521	0.592
	Width of the fruit (cm)	50	6.87	0.60		0.942	0.686
	Thickness of the fruit (cm)	50	6,68	0.61		0.091	0.930
	Mass of the mesocarp (g)	50	166.24	42.79		0.456	0.413
	Mass of the pit (g)	50	5.68	1.73		0.001**	0.001**
	Yield (%)	50	95.35	0.46		0.000**	0.000**
2012	Mass of the fruit (g)	50	170.18	38.17	0.586		0.883
	Height of the fruit (cm)	50	6.36	0.44	0,521		0.915
	Width of the fruit (cm)	50	6.88	0.54	0.942		0.634
	Thickness of the fruit (cm)	50	6.87	0.59	0.091		0.109
	Mass of the mesocarp (g)	50	160.90	36.40	0.456		0.941
	Mass of the pit (g)	50	6.74	1.83	0.001**		0.940
	Yield (%)	50	94.52	0.30	0.000**		0.000**
2013	Mass of the fruit (g)	50	169.08	26.99	0.489	0.883	
	Height of the fruit (cm)	50	6.35	0.43	0.592	0,915	
	Width of the fruit (cm)	50	6.83	0.41	0.686	0.634	
	Thickness of the fruit (cm)	50	6.69	0.49	0.930	0.109	
	Mass of the mesocarp (g)	50	160.37	25.88	0.413	0.941	
	Mass of the pit (g)	50	6.76	1.22	0.001**	0.940	
	Yield (%)	50	94.83	0.33	0.000**	0.000**	

n - number of examined fruits; $\bar{x}$ - average value; SD - standard deviation; p - statistical significance, * Significant differences at the significance level of 0.05 ($p<0.05$); ** Significant differences at the significance level of 0.01 ($p<0.01$).

Lower values for mass of the peach fruits from the cultivar ‘Cresthaven’, cultivated in different locations in Serbia in the period 2000-2003 were found by Zec et al. (2003). The authors found that the mass of the fruits of this variety cultivated at Padinska Skela has average values

ranged from 59.90 g to 110.60 g, and at Grocka location from 57.30 g to 126.00 g.
The following mean values expressed in cm for height, width and thickness of the fruit in each of the years vary from 6.30 ± 0.55 to 6.36 ± 0.44, from 6.83 ± 0.41 to 6.88 ± 0.54 and from 6.68 ± 0.61 to 6.87 ± 0.59. From Table 1 can also be determined that the mass of mesocrap is from 160.37 ± 25.88 to 166.24 ± 42.79 g, and the pit mass from 5.68 ± 1.73 to 6.76 ± 1.22 g. The calculated values of the yield are ranging from 94.52 ± 0.30 to 95.35 ± 0.45%.
A comparison of the results from the examinations of the peach fruits from the cultivar 'Cresthaven', by years, indicates the existence of certain differences in the calculated values of the examined parameters. With statistical analysis it has been determined their significance (Table 1).
In terms of mass of fruits in each of the years under examination, it can be concluded that in 2012 and 2013 were obtained lower values (170.18 g and 169.08 g) compared to 2011 (174.24 g). With the statistical analysis of the data it has been determined that these differences are not statistically significant ($p>0.05$).
In terms of heigh (6.30 cm, 6.36 cm, 6.35 cm) and width (6.87 cm, 6.88 cm, 6.83 cm) of fruits it was found that the differences between the values obtained for this period are not statistically significant ($p>0.05$).
Small differences in the values obtained have been determined for the thickness of the fruit in 2011, 2012 and 2013 (6.68 cm, 6.87 cm, 6.69 cm), also confirmed by a statistical standpoint ($p>0.05$). Also, there were identified some variabilities in values obtained for mass of mesocrap, which proved that there was no statistically significant difference ($p>0.05$).
For pit mass of the fruits have been obtained approximations in 2012 and 2013 (6.74 g, and 6.76 g). The little difference which occurs in the values obtained for this parameter is not statistically significant ($p>0.05$). In terms of pit mass of the fruits in 2011 it has been determined that it is smaller (5.68 g) than the mass of pit in 2012 and 2013. Statistical analysis of the data has shown that differences which occur between the values of pit mass in 2011 and 2012 and between 2011 and 2013 are statistically significant ($p<0.01$).
The yield is closely connected with the mechanical composition of raw materials (Niketić-Aleksić, 1994). The differences which appeared in the pit mass of fruits affected the obtained values of the yield. Statistical analysis of the results for the yield of fruits has shown statistically significant differences ($p<0.01$).
From Figure 2 it can be concluded that the average mass of the fruits from the cultivar 'Cresthaven' for the three year period is 171.17 g, height 6.34 cm, width 6.86 cm, and thickness of 6.75 cm. The average mass of the fleshy interior is 162.50 g, the average pit mass is 6.39 g, and the yield 94.90%.

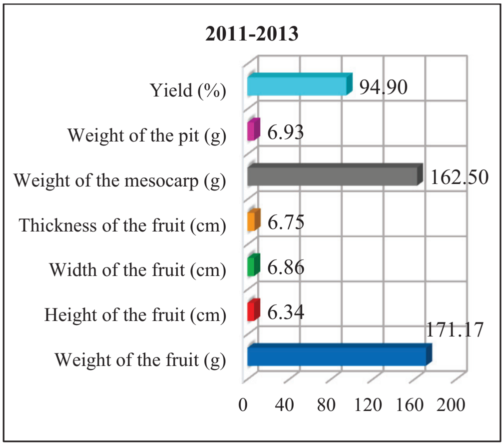


Figure 2. Pomological characteristics of fruits of peach cultivar 'Cresthaven' for all three years

Compared with the results obtained from our research, lower values for the same parameters are presented by Zec et al. (2003). Namely, the three-year research period of peach fruits from the cultivar 'Cresthaven' it has been determined that in average they are characterized by a mass of 92.8 g, a height of 5.41 cm, a width of 5.67 cm and a thickness of 5.37 cm. Higher average mass value (117.00 g) is determined by the same author in 2005 in a two-year examination of the peach fruits from the cultivar Cresthevn cultivated at the locations of Mislodzhin and Padinska Skela, but this average value is lower than the value obtained from our determinations.
Contrary to previous results Nenadović-Mratinić et al. (2003) for the tested parameters have evidenced slightly higher average values of the values obtained from our research. In seven years period of examining peach fruits

from the cultivar 'Cresthaven' they have concluded that this variety of peach fruits is featured with a mass of fruit of 184.00 g, a length of 6.75 cm, a width of 7.41 cm, thickness of 7.56 cm, mass of pit 8.09 g and yield of 95.60%.

Differences between the results obtained from the research and the previously mentioned bibliography data are expected, because, although it is a fruit of the same variety of peach, the conditions under which it has cultivated are different.

Heavy metals are defined as metals whose specific density is greater than 5 g/cm^3. They affect human health through pollution of the environment and through the food, mainly through the fruits and vegetables cultivated in soil that has been contaminated. These include: arsenic, cadmium, chromium, copper, lead, nickel, zinc, molybdenum and vanadium (Islam, 2013).

During the research it has been conducted an analysis for the presence of Pb, As and Cd in the fruits of the cultivar 'Cresthaven'. The obtained results of the conducted analyzes in the three years of testing are shown in Table 2.

Table 2. Content of Pb, As and Cd in peach fruits of the cultivar 'Cresthaven' (2011, 2012, 2013)

Heavy metals / Year	Pb mg/kg (L)	As mg/kg (L)	Cd mg/kg (L)
2011*	0.008	0.000	0.000
2012*	0.021	0.000	0.006
2013**	0.023	<0.001	0.002

*According to Regulation on general requirements for food safety (Official Gazette of RM No.118/2005).

** According to Regulation on the general requirements for food safety in relation to the maximum levels of certain contaminants (Official Gazette of RM No.102/2013).

From the submitted results it can be found that in the three years of examining the content of Pb, As and Cd in the peach fruits from the cultivar 'Cresthaven' is in accordance with the prescribed standards for food safety (Regulation on general requirements for food safety, Official Gazette of RM No.118/2005; Regulation on the general requirements for food safety in relation to the maximum levels of certain contaminants, Official Gazette of RM No.102/2013).

CONCLUSIONS

Based on three-year research period for the peach fruits of the cultivar 'Cresthaven' cultivated in Rosoman, Macedonia can be concluded that they were characterized by features inherent for their type and variety.

From a statistical point of view between the calculated values of the tested parameters there are statistically significant differences between the years of testing, in terms of pit mass and the yield.

From the calculated values for the content of Pb, As and Cd in fruits in the three years of testing it can be concluded that they are in accordance with the prescribed standards for food safety and are safe for consumption.

It is expected that the results obtained will find applications for the primary producers and direct consumers, which will increase the placement of this kind of fruit in the domestic and foreign market.

REFERENCES

Bulatović-Danilović M., 2007. Peach. Manual for the production of peaches for fresh use. Ministry of Agriculture, Forestry and Water Economy of the Republic of Serbia, Belgrade.

Chandorkar S., Deota P., 2013. Heavy metal content of foods and health risk assessment in the study population of Vadodara. Current World Environment, 8(2): 291-297.

Crisosto H.C., Valero D., 2008. Preharvest factors affecting peach quality. In: Layne D. R., Bassi D. (Eds.), The peach, Botany, Production and uses, (Edited by), London, UK: CAB International, 536-550.

Elbagermi A. M., Edwards M. G.H., Alajtal I. A., 2012. Monitoring of heavy metal content in fruits and vegetables collected from production and market sites in the Misurata Area of Libya. International Scholarly Research Network, ISRN Analytical Chemistry, Volume 2012, Article ID 827645, 5 pages, doi:10.5402/2012/827645.

Ghazanfarirad N., Dehghan K., Fakhernia M., Rahmanpour F., Bolouki M., Zeynali F., Asadzadeh J., Bahmani M., 2014. Determination of lead, cadmium and arsenic metals in imported rice into the West Azerbaijan Province, Northwest of Iran. J Nov. Appl Sci., 3(5): 452-456.

Hajilou J., Fakhimrezaei S., Dehghan G., 2013. Fruit quality, bioactive compounds and antioxidant capacity of 6 Iranian peach cultivars, Research in plant biology, 3(6): 06-16.

Islam R., 2013. Consumption of unsafe foods: Evidence from heavy metal, mineral and trace element

contamination. Department of Soil Science Bangladesh Agricultural University, Mymensingh.
Jašić M., 2007. Technology of fruit and vegetables. Part 1. University of Tuzla, Faculty of Technology, Tuzla.
Kantoci D., 2008. Stone fruit. Bulletin of plant protection, 31(5): 6-13.
Karakašova Lj., 2011. Processing of fruits and vegetables. University St. Cyril and Methodius - Skopje, Faculty for Agricultural Sciences and Food, Skopje.
Karakašova Lj., Babanovska-Milenkovska F., 2012. Processing of fruits and vegetables – Handbook. University St. Cyril and Methodius - Skopje, Faculty for Agricultural Sciences and Food, Skopje.
Matei N., Popescu A., Munteanu M., Gabriel Lucian, Radu L. G., 2013. The assessment of Cd, Zn, Pb, Cu and vitamin C in peaches. U.P.B. Sci. Bull., Series B, 75(1): 73-80.
Milošević T., Milošević N., 2011. Quantitative analysis of the main biological and fruit quality traits of F1 plum genotypes (*Prunus domestica* L.), Acta Sci. Pol., Hortorum Cultus 10(2): 95-107.
Mratinić E., 2000. Peach - Nectarine. Agricultural library Draganć, Belgrade.
Nenadović-Mratinić E., Milatović D., Đurović D., 2003. Biological – economic characteristics of peach cultivars of mid-season to late ripening time, Proceedings of scientific papers, 9: 169-175.
Niketić-Aleksić G., 1994. Technology of fruit and vegetables. III Edition. Faculty of Agriculture, Belgrade.
Nikolić D., Rakonjac V., Fotirić-Akšić M., Radović A., 2013. Characteristics of peach hybrids from the crossing combination Flaminia × Hale Tardiva Spadoni, Proceedings of the IV Conference "Innovations in Fruit Growing“, Belgrade, 197-207.
Regulation on general requirements for food safety, Official Gazette of RM No.118/2005.
Regulation on the general requirements for food safety in relation to the maximum levels of certain contaminants, Official Gazette of RM No.102/2013.
Sobukola P. O., Adeniran M. O., Odedairo A. A., Kajihausa E. O., 2010. Heavy metal levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. African Journal of Food Science, 4(2): 389 – 393.
Stojmilov A., Apostolovska-Toševska B., 2016. Socio-economic geography of the Republic of Macedonia. University St. Cyril and Methodius – Skopje, Faculty of Natural Sciences and Mathematics, Skopje.
Vuletić T., 2016. Biological characteristics of peach (*Prunus persica* (L.) Batsch) in modern farming. Final work. University of Zadar, Department of ecology, agronomy and aquaculture, Zadar.
Zec G., Čolić S., Mišić P., Todorović R., Marinković D., 2003. Cv Gročanka – A new late peach cultivar, Jugosl. Voćar, 37 (143-144): 105-112.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

AN *IN VITRO* STUDY OF COMMERCIAL FUNGICIDE EFFECTS ON POLLEN GERMINATION IN APPLE

Sultan Filiz GUCLU[1], Fatma KOYUNCU[2]

[1]Süleyman Demirel University, Atabey Vocational School, Isparta, Turkey
[2]Süleyman Demirel University, Agricultural Faculty, Isparta, Turkey

Corresponding author email: sultanguclu@sdu.edu.tr

Abstract

In this study some fungicides effects (Captan, azoxystrobin, mycolobutanil, thiophanate - methyl, maneb) on pollen viability tests and in vitro pollen germination investigated in Red Chief's pollens. Pollen viability test was made by TTC (2, 3, 5-triphenyl tetrazolium chloride) 15% +0.5% agar-agar+5 ppm boric acid at 25^{0}C medium as control medium. Pollen germination was conducted at three concentrations: the recommended fields rate (100% RFR), 10% RFR and 1% RFR of each fungicide. 'Agar in plate' method was used for pollen germination tests. Statistical analyses performed with GLM models Using SPSS. Pollen germination rate was inhibited by increasing doses of fungicides when compared with control medium. Captan and azoxystrobin were most inhibitory. Germination was not significantly affected by mycolobutanil. Also Thiophanate –methyl was found inhibitory.

Key words: *pollen germination, pesticide, 'Red Chief'.*

INTRODUCTION

Rapidly increasing world population is regarded as one of the most important problems for mankind in the decades to come. A decline in the agricultural areas plays an important role in this direction and lead to certain restrictions in feeding the growing population. Therefore, an evaluation of the available land for obtaining the maximum yield has become a major goal. One of the applications in this connection has been the use of pesticides in plants against harmful organism for improving agricultural productivity. However, in addition to the benefits they provide, an excessive use of these chemicals, wrong applications as well as their side-effects have started creating serious environmental problems as well as posing toxicity threat to living organisms.

Inhibitory effects of pesticidal sprays during pollination would be of particular concern in areas where pollination and fertilization are limiting factors in fruit production. Recently, there has been a lot of press related to pollinator health, and some troubling information indicates that certain fungicides, when used during bloom, can negatively affect the health of honey bees. This is a complicated problem with the solutions relying on understanding the detailed relationships among chemicals, pollinators and pest management needs. The objective of this study was to evaluate the effects of selected fungicides from different class.

MATERIALS AND METHODS

Plant material

Red Chief's pollens were used for pollen tests. Pollens were obtained from flowers of the above mentioned at balloon stage. The flowers were transferred to the laboratory immediately. Anthers were removed and placed into the dark-colored bottle to promote dehiscence at room temperature. The fungicides, which were commonly used for apple in Isparta selected for tests.

Table 1. Used fungicides

Active ingredient	Class	Trade name	Formulation*
Captan	Dicarboximide	Captan	WP
Azoxystrobin	Strobilurin	Quatris	WG
Mycolobutanil	Azole	Rally	WSP
Thio phanate-methyl	Benzimidazole	Topsin M	WSP
Maneb	Dithiocarbomate	Manex	Flowable

*WP=wettable powder, WG=water-dispersable granule, WSP=water-soluble pouches

The class, trade name, formulation and recommended field rate for each of compounds are shown in Table 1. Applies were made according to Yi et al., 2003. Pollen germination and tube growth were conducted at three concentrations: the recommended fiels rate (100% RFR), 10% RFR and 1% RFR of each 'fungicide'. The pollen morphological homogeneity percentages of pollens were assessed with thehaemocytometer (Marienfeld, Germany) slide (Eti, 1990). Imperfectly shaped pollen grains were considered as aborted pollen. The final percentage of morphological homogeneity was defined as:

(number of normal shaped pollen) – (number of aborded pollen) per field

MH = total number of pollen field × 100

Pollen viability was determined by TTC (2, 3, 5-triphenyl tetrazolium chloride) stain test. Pollens were scattered onto TTC and stained pollens were counted after 2 hours and 15 minutes, respectively.

To determine the pollen viability, pollens of Red Chief apple (of four different areas) were observed onto two slides under a light microscope (×100 magnification). The stained pollen was considered as viable in these tests.

Germination medium without fungicide served as control.

For the *in vitro* test, pollen grains were sowed in the medium containing 0.5 agar + 15% sucrose + 5 ppm H_3BO_3 (boric acid) and incubated at the constant temperature of 25°C. The 'agar in plate' method was used to assisgn pollen germination and pollen tube growth (Koyuncu and Güçlü, 2009).

An ocular micrometer was used to measure pollen tube lenght, under a light microscope, at a magnification.

Four Petri dishes were used for germination and pollen tube growth experiments. For each assay, 2mL of medium with or without 'fungicide' was placed into Petri dishes.

Counts were made from 4 different microscope fields (100-150 pollen grains per field for each Petri dishes) (Hedly et al., 2004; Koyuncu, 2006).

A factorial design was used in this study. Each treatment (5 'fungicide's x 3 concentrations plus control) was replicated 4 times.

Statistical analysis was conducted using Duncan's multiple range test within the general linear model procedure of SPSS 16.0.

For germination percentage, data were transformed with arcsine to meet the equal variance assumption.

RESULTS AND DISCUSSIONS

Pollens were evaluated from morphological homogeneity and viability there is no significant effect comperad to the control group. (Table 2).

Table 2. Morphological homegeneity and viability ratios (%)

	Morphological homogeneity (%)	Staining Test TTC (%)
Control (No fungicide)	98	97
Captan	96	96
Maneb	94	92
Mycolobutanil	93	90
Thiophanate-methyl	94	92
Azoxystrobin	92	90

Morphological homogeneity and viability rates were obtained upper 90%. However the lightest colour pollens were observed from Azoxystrobin but they were adopted 'viable'. In this case, the azoxystrobin fungicide may impair the structure of the enzyme when exposed to long-term or ex suggesting potential damage layer.

Different staining tests were carried out different fruit cultivars by different researches. Tosun and Koyuncu 2007, studied on cherry pollens, Koyuncu (2006), studied strawberry pollens using TTC and reported that pollen viability ratios reached 82% (Allstar and Elvira) and 86,5% (Chandler).

There are a lot of studies different fruit species about pollen viability tests. Junqueira, 2016 pointed that the viability of pollen grains was affected by the application of fungicide P + E, regardless of the application time. On the other hand, pollen grain germination was not affected by the fungicide or the stage.

Azoxystrobin and captan remained extremely inhibitory, with germination less than 1% of control. Maneb also inhibitory effect of pollen germination with 13.7%.

The highest pollen germination obtained from medium which was contain mycolobutanil with 87.7%. Also thiophanate-methyl have 68.8% germination. The inhibitory effects of some fungicides have been reported different study. Especially in apple, Yi et al 2003 reported the pollen germination in apples treated with Captan decreases by 20 % as compared to the control. Also captan was reported the inhibit pollen germination in pear (Butt et al., 1985).

The new azole product, mycolobutanil, had little or no inhibition, while the other new product, azoxystrobin, had very toxic effects (Yi et al., 2003b). Parallel these findings from our study captan and azoxystrobin were found severely inhibitor for pollen germination. After then maneb has found inhibitory for pollen germination. Mycolobutanil had little inhibition.

A number of compounds tested included fungicides different chemical class, i.e., dicarbomixide, strobilurin, azole, benzimidazole, dithiocarbomates were evalueted. The benzimidazo (thiophanate-methyl) had no to-intermediate effects. The dithiocarbamate compound (maneb) more severely suppressed pollen germination. Azoxystrobin, a strobilurin, was highly inhibitory. shown Table 3.

Table 3 Germination of apple pollen in presence of selected fungicides

Fungicides	Fungicide conc. (%of RFR)		
	100	10	1
Control (No 'fungicide')	100a[y]	100a	100a
Captan	0.0b	0d	0.9e
Maneb	0.0b	0.0d	13.7d
Mycolobutanil	0.0b	46.9b	87.7b
Thiophanate-methyl	0.0b	10.2c	68.8c
Azoxystrobin	0.0b	0d	0.3e

[z]Germination percentages shown are relative to the control, which is expressed at 100%. Actual pollen germination in the no-'fungicide' control was 77.4%. RFR=recommenden field rate

[y] Mean values within a column followed by the same letter are not significantly different at p=0.05, Duncan's multiple range test.

With 88.6% pollen germination rate obtained from the control medium (0.5 agar + 15% sucrose + 5 ppm H_3BO_3).

There is no pollen germination was observed in assays incorporatting any of the fungicides at 100% RFR (Recommended field rate).

Pollen grains typically exhibit high sensitivity to chemicals with *in vitro* germination assays where contact with chemicals is intense. Although germination was inhibited severely at 10% RFR, the fungicides showed differential effects as pollen germination was observed in the presence of maneb, captan and azoxytrobin. Germination in thiophanate- methyl was only 10.2%.

The highest relative germination occured in the presence of mycolobutanil which was 46.9% of control. Assays conducted at 1% were effective in delineating differences in polen sensitivity to different fungicides. Found that fungicide sprays caused detrimental effects on stigma morphology and enhanced exudates production in almond flowers.

Percent fruitset was not measured in the study, however increased exudates production was raised as possibly causing inhibition of pollen tube growth and germination. It was also suggested that the increased exudates production may be a stress response which could decrease the period of stigma receptivity (Yi et al., 2003). Cyprodinil promoted a copious increase in exudates secretion and caused the most severe collapse of stigmatic cells of all the fungicides evaluated in the almond study.

Fungicides incorporated into the media, or sprayed on the surface of the medium, reduced pollen germination and pollen tube growth at concentrations lower than those commercially recommended for successful disease control (Heazlewood, 2004).

The mode of action of the fungicide, systemic or contact, is thought to alter the level of damage caused to pollination.

It should be noted that effects of 'fungicide's on pollen under in vio conditions will be affected by additional considerations, such as the persistence of the chemical, whether orr not it is systemic, and how it may interact with the constituents of the stigmatic papilae (Yi et al., 2003).

CONCLUSIONS

Commercial 'fungicide's haven't affected so much to morphological homogeneity and pollen viability. Pollen viability rates changed between 90% and 97%. Pollen germination rates decreased by incresing doses of all 'fungicide's.

All 'fungicide's must have used recommended dose. Azoxystrobin was found the most dangerous.

REFERENCES

Butt D.J., Swait A.A. and Robinson, J. D., 1985. *Effect of fungicides on germination of apple and pear pollen*. Ann. Appl. Biol. 106 Suppl: 110-111.

Eti S., 1990. *Cicek tozu miktarini belirlemede kullanilan pratik bir yontem*. Journal of Agricultural Faculty, Cukurova University, 5: 49–58.

Heazlewood J. E. Wilson. Clark S. R. J and Gracıe A. 2005. *Pollination of Vitis vinifera L. cv. Pinot noir as* *influenced by Botrytis fungicides*. Vitis 44 (3), 111–115

Hedly A.. Hormaza J.I and Herrero M., 2004. *Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, Prunus avium (Rosaceae).* Am J Bot 91 (4), 558-564.

Junqueria B.V., Costa C.A., Boff T., Muller C., Mendonça C.A.M., Batista F.P., 2016. *Pollen Viability, Physiology and production of Maize Plants Exposed To Pyrocloctrobin+epoxiconozole*. Pesticide Biochemistry and Physiology, YPEST-03991htpp://dx.doi.org/1016

Koyuncu F., 2006. *Response of in vitro pollen tube growth of strawberry cultivars to temperature.* European Journal of Horticultural Science, 71: 125–128

Koyuncu F. and. Tosun (Guclu) F. 2009. *Evaluation of pollen viability and germinating capacity of some sweet cherry cultivars grown in Isparta, Turkey*. Acta Hort.795 ISHS, (1), 71-75.

Tosun (Güçlü), F. and Koyuncu F., 2007. *Investigations of suitable pollinator for 0900 Ziraat sweet cherry cv.: pollen performance tests, germination tests, germination procedures, in vitro and in vivo pollinations*. HortSci(Prague), 34, (2), 47-53

Yı W., S.E. Law and H.Y. Wetzstein, 2003a . *Pollen tube growth in styles of apple and almond flowers after spraying with pesticides.* J. of Hortic. Sci. and Biotec., 78(6): 842-846.

Yi Weiguang, Law S. E., Wetzstein H., Y., 2003b. *An in vitro study of fungicide effects of pollen germination and tube growth in almond*. HortScience 38 (6):1086-1088.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE INFLUENCE OF STORAGE IN CONTROLLED ATMOSPHERE ON QUALITY INDICATORS OF THREE BLUEBERRIES VARIETIES

Ioana BEZDADEA CĂTUNEANU[1,2], Liliana BĂDULESCU[1,2], Aurora DOBRIN[2], Andreea STAN[2], Dorel HOZA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Marasti Blvd., District 1, Bucharest, Romania
[2] University of Agronomic Sciences and Veterinary Medicine of Bucharest, Research Center for Studies of Food Quality and Agricultural Products,
59 Marasti Blvd., 011464, Bucharest, Romania

Corresponding author email: ioana.catuneanu@gmail.com

Abstract

The aim of this study was to determine which storage conditions can preserve the blueberry quality (Vaccinium corymbosum L.), stored in three different rooms with controlled atmosphere (CA). For this purpose, three varieties of blueberries, like Coville, Blueray and Chandler were stored and monitored for four months. Quality parameters like: dry matter content (D.M.%), titratable acidity (TA), soluble solids (°Brix), firmness, antioxidant capacity and also content in flavonoids, total polyphenols, total anthocyanins and ascorbic acid was monitored during storage period. The experiment conditions were based on the variation of carbon dioxide (CO_2) as follows: Room 1 (CO_2: 0%, representing the control), Room 2 (CO_2: 5%), and Room 3 (CO_2: 10%). Other common parameters of the experiment were: temperature (t°) 1 °C, oxygen quantity (O_2) 3%, relative humidity (RH) 95%. After four months of storage, observations showed that blueberries from Chandler variety presented better quality parameters compared to blueberries from Coville and Blueray varieties. Moreover, notable differences of physical and biochemical parameters were observed within the same blueberries variety stored in different rooms with controlled atmosphere conditions. Blueberries stored in Room 2 (T: 1°C, O_2: 3%, CO_2: 5%, RH: 95%) and Room 3 (T: 1°C, O_2: 3%, CO_2: 10%, RH: 95%) presented the best quality attributes compared with those stored in the other storage room (control), which would translate to a longer shelf life.

***Key words**: blueberries, controlled atmosphere, storage, quality.*

INTRODUCTION

Since the Neolithic, blueberries (*Vaccinium* spp.) were consumed (Wang et al., 2017) at the beginning due to their wonderful taste sweet and sour and after centuries also for their biochemical composition (Wang et al., 2017) and health benefits (Liato et al.,2016). For this reason the production and consumption of blueberries has increased yearly and in recent years, they became one of the most popular horticultural products all over the world, second only after strawberries (Chen et al.,2015). They are sold fresh, processed, and in frozen form for various applications in food retail markets (Yang et al., 2014). Blueberries are appreciated for their taste, their high antioxidant activities, high and rich bioactive level contain of vitamins (C and E) (Liato et al.,2016), anthocyanins (Xu et al., 2016), polyphenolics (Liato et al.,2016), acids, tannins, mineral elements (Xu et al.,2016), chlorogenic acid, procyanidins (Chen et al., 2014) and flavonols (Wang et al., 2017).
Blueberries have antioxidant, anti-inflammatory, antimicrobial, anti-proliferative actions and they can be used in: type 2 diabetes (Shi et al., 2017), diabetic retinopathy (Song et al.,2016), cardiovascular and neuro-vegetative diseases, cancer (Liato et al.,2016), arthritis and obesity (Shi et al.,2017).
Due the increased production of fresh blueberries (Liato et al, 2017) from all over the world, a very important aspect is assuring and maintaining nutritional quality, and microbiological safety during storage and post-harvest sales (Liato et al.,2016).
Liato (2017) suggested that 95% of the blueberries production exhibit fungal contamination. Yang (2014) noticed that fresh blueberries rapidly deteriorate due to water loss and degradation of the fruit, usually caused by

fungi such as: Anthracnose (*Colletotrichum acutatum*), Alternaria (*Alternaria* spp.) and grey mold (*Botrytis cinerea*) (Yang et al., 2014). According to Chen (2015) fresh blueberries are highly perishable and they have between 1 and 8 weeks of shelf life, so it is very important how the methods of harvesting, storage and transport conditions are applied. Varela (2008) studied how long controlled atmosphere storage prolong shelf life of apples, until consumption with the following storage conditions: T=1^{o}C, O_2=2% and CO_2=2%, and the result was 7 months. Also it was observed that the loss of firmness is closely related to changes in cell wall composition and decrease in the total water soluble pectin (Chen et al., 2015). Firmness loss, which seriously reduces the commercial value of blueberries (Xu et al., 2016), can be slowed down by a bioactive, biocompatible and biodegradable polysaccharide such as chitosan (Yang et al., 2014). It acts as a barrier of water vapour to reduce the damage, reducing the loss firmness, decreasing mould growth and extending shelf-life (Yang et al., 2014) of strawberries and blueberries. Fungi can be significantly reduced by limited O_2 and increased CO_2 levels in the package (Yang et al.,2014) and in this way the quality of the texture can be maintained during storage (Chen et al.,2015). However, as Bessemans (2016) observed, the blueberries created storage disorders and an off-flavor by low oxygen content in rooms with controlled atmosphere, but Cortellino (2017) showed that firmness of the apples has been maintained better in low oxigen conditions. Francini (2013) suggests that the content of total phenols in apples had decreased during cold storage.

It was observed that the postharvest preservation technologies are applied to reduce damage, prolong shelf life, and keep the nutritional quality of several fruits and vegetables (Liato et al., 2017). Some of postharvest preservation technologies that can be used are: cold room storage, edible coatings, UV irradiation (Xu et al.,2016), packaging in a modified atmosphere (Yang et al.,2014), ozonation, and fumigation of sulphur dioxide (Yang et al.,2014), chlorine dioxide (Xu et al.,2016) (ClO_2 - strong oxidizing and sterilizing power).

The aim of these study was to determine which of the storage conditions of three different rooms with controlled atmosphere (CA), can better preserve the quality of blueberries (*Vaccinium corymbosum* L.).

MATERIALS AND METHODS

Fruits sampling and preparation

In order to accomplish the aim of this study three blueberry varieties (*Vaccinium corymbosum* L.) were used: Coville, Blueray and Chandler. These were acquired in August 2016 from Bilcesti, (Arges, Romania) and were selected by commercial maturity and the same ripening stage of fruits. Blueberries were packed in 250 g perforated trays that were then stored in three rooms with controlled atmosphere conditions from the Research Center for Studies of Food Quality and Agricultural Products - University of Agronomic Sciences and Veterinary Medicine of Bucharest. Temperature (t°) 1°C, oxygen (O_2) 3% and relative humidity (RH%) 95% was the same for all three rooms, but the CO_2 level was different. Thus, in room 1, which represents the control, the CO_2 concentration was 0%, CO_2 concentration in room 2 was 5%, and 10% in room 3 (Rizzolo et al., 2010). The study was conducted in 4 different moments as follows: initial moment (0), after 2, 3 and 4 months of storage in controlled atmosphere (CA). All samples were performed in duplicates.

For total flavonoid, total polyphenol and antiradical activity, the blueberry samples were extracted in 50% ethanol. For total anthocyanin acidified methanol was used (1.0% (v/v) hydrochloric acid in methanol) and for ascorbic acid the samples (5 g each) were extracted in 50 ml 9% metaphosphoric acid (MPA).

Physico-chemical analysis

The ***dry matter and water content*** of the samples were determined by oven drying for 24 hours at 105^{o}C using a UN110 Memmert oven, method used also by Moura (2005), Skupień (2006), Delian (2011), Corollaro (2014), Mureşan (2014), Ticha (2015). To determine the fruit firmness an electronic penetrometer

TR was used, and the results were expressed in kg/cm^2 (Chen, 2015).

Soluble solids were determined from blueberry juice (Yoon, 2005; Saei, 2011; Mureşan, 2014; Oltenacu, 2015), with refractive device Kruss DR301-95 (% Brix). The titratable acidity was determined by titration with 0.1N NaOH to pH 8.1 (DeEll, 1992; Yoon, 2005; Skupień, 2006; Saei, 2011).

Titratable acidity calculation was done using the formula: $\frac{F \times C \times a \times b \times 100}{b \times c}$, where F is the factor NaOH solution 0.1 N (1,002), C = coefficient of correction for citric acid (0.0064), a = quantity of 0.1 N NaOH titrated, b = volume of the extraction solution, c = mass of the sample. For titration with 0.1 N NaOH the automatic titrator TitroLine easy was used. The results were expressed in g citric acid/100g.

Total flavonoid content was determined after an aluminium chloride adapted method (Žilić, 2011; Shen, 2016; Li, 2017). 0.25 ml hydro-alcoholic extract was mixed with 1.25 ml of distilled H_2O and 0.075 ml of a 5% $NaNO_2$, after five minutes 0.075 ml of a 10% solution of $AlCl_3$ was added. After another six minutes 0.5 mL of 1M solution of NaOH was added, the final volume being 2.5 ml. The absorbance was read at wavelength λ = 510 nm. The total flavonoid content was expressed in M/ml in fresh weight.

Total polyphenol content was measured by colorimetric Folin-Ciocateu method after Skupień (2006), Khanizadeh (2008), Delian (2011), Mureşan (2014) and Drogoudi (2016), with some modification. 25 µl of a hydro-alcoholic extract were made up to 2 ml with distilled H_2O. 125 µl Folin - Ciocalteu and 375 µl of Na_2CO_3 (used for an alkaine environment) was added to the mixture. The final volume was 2.5 ml. The wavelength used for mesurements was λ = 750 nm. The total polyphenol content was expressed in M/ml in fresh weight.

Total anthocyanins content was measured with spectrophotometric absorbance at wavelength λ = 540 nm (Bărăscu et al., 2016), after an adapted method. The extracts were filtered under vacuum and completed up to 50 ml volume. The results were calculated using the formula: Total anthocyanins = DO_{540} x F, where DO_{540} is absorbance at wavelength λ = 540 nm and factor F = 11.16. The total anthocyanins content was expressed in mg/100g in fresh weight.

For evaluation of ***antiradical activity*** an indirect DPPH-radical scavenging activity spectrophotometric method was used (Khanizadeh, 2008; Mureşan, 2014; Drogoudi, 2016). 0.5 ml hydro-alcoholic extract was mixed with 1 ml of 0.1 mM DPPH solution. The results were calculated using the formula: AA_{DPPH} (%) = $\frac{A\ control - A\ sample}{A\ control}$ x 100, where $A_{control}$ is absorbing control sample (containing all reagents except extract) and A_{sample} is the sample absorbance. The absorbance was measured at wavelength λ = 515 nm. The evaluation of antiradical activity was expressed in % in fresh weight.

All determinations described above were performed with Specord 210 Plus spectrophotometer.

Ascorbic acid content was determined with HPLC – Agilent Technologies 1200 Series equipment, using an ZORBAX Eclipse XDB-C18 (4.6x50 mm, 1.8µm) column with Rapid Resolution HT and a detector UV-DAD detection wavelength 220/30 nm, reference wavelength 400/100 nm. Mobile phases were A= 99% (ultrapure water with H_2SO_4 up to 2.1 pH) and B= 1% (acetonitrile with 10% A). The samples were filtered through a filter Agilent PTFE 0,2 µm. The injection volume was 2 µl, with 4 min post time, flow rate at 0.5 ml/min at 30 ^{o}C in column compartment. The samples were analysed in duplicate and were expressed in mg/100g. Ascorbic acid calculation was done using the formula: $\frac{a \times b \times 100}{c}$, where a=ascorbic acid content in mg/ml , b= solution extraction volume (ml) and c= working mass of the sample taken (g).

RESULTS AND DISCUSSIONS

Blueberries fruit quality is assessed by the following indicators: firmness, dry matter, water content, soluble solids and titratable acidity. The quality indicators were different both at harvest and during storage for all the blueberry varieties.

The dry matter and water content of Coville variety had small variations during storage compared to initial moment (0) (Table 1), observing more fluctuations in the control room (CO_2 - 0%).

The water content and the titratable acidity (TA) had the highest value after three month of storage in the room 2 (CO_2 - 5%).

For Coville variety stored in room 1 (control) the soluble solids content had the maximum value after two months of storage and the dry matter and firmness after three months of storage compared to the other two rooms.

Table 1.Variation of firmness and content of: dry matter, water, TA and soluble solids during storage period in CA for Coville variety

Sample	Time of analysis	Dry matter content(D.M.%)	Water content (%)	Titratable acidity (g acid citric/100 g)	Soluble solids (% Brix)	Firmness (kg/cm^2)
Coville	17.08.2016	14.334	85.666	1.016 ± 0.009	10.420 ± 0.751	0.294 ± 0.032
Coville room1	13.10.2016	13.870	86.130	1.087 ± 0.002	11.670 ± 1.063	0.383 ± 0.103
Coville room2	13.10.2016	14.366	85.634	1.061 ± 0.001	11.160 ± 1.251	0.293 ± 0.071
Coville room3	13.10.2016	14.256	85.744	0.889 ± 0.005	9.920 ± 1.396	0.255 ± 0.042
Coville room1	22.11.2016	15.121	84.879	1.136 ± 0.021	10.820 ± 1.434	0.384 ± 0.062
Coville room2	22.11.2016	13.217	86.783	1.302 ± 0.005	10.660 ± 2.219	0.333 ± 0.063
Coville room3	22.11.2016	13.778	86.222	1.092 ± 0.003	9.440 ± 1.608	0.329 ± 0.092
Coville room 1	12.12.2016	14.907	85.093	0.967 ± 0.022	10.470 ± 1.589	0.341 ± 0.074
Coville room 2	12.12.2016	14.794	85.206	1.161 ± 0.001	10.570 ± 1.455	0.257 ± 0.044
Coville room 3	12.12.2016	13.829	86.171	1.105 ± 0.001	10.280 ± 1.605	0.268 ± 0.090

In table 2, for Blueray variety, it has been observed that the content of dry matter and water had some variation during storage in comparison with the initial moment.

Blueray variety stored in rooms 1 and 2 have lost turgidity towards the end of storage period. Titratable acidity (TA) and soluble solids content have different values during storage, compared to initial moment (0). The soluble solids content from room 3 (CO_2 - 10%) and titratable acidity in all 3 rooms registered an increase in comparison with the initial moment (0) (Yang et al., 2014). Also, there was an increase in firmness in room 1 (CO_2 - 0%) after two months of storage. In room 2 and 3 the firmness value was close to the initial moment (0), the maxim firmness of the fruits was recorded after three months of storage.

Table 2.Variation of firmness and content of: dry matter, water, TA and soluble solids during storage period in CA for Blueray variety

Sample	Time of analysis	Dry matter content(D.M.%)	Water content (%)	Titratable acidity (g acid citric/100 g)	Soluble solids (% Brix)	Firmness (kg/cm^2)
Blueray	17.08.2016	11.458	88.542	0.620 ± 0.009	8.400 ± 1.339	0.243± 0.063
Blueray room 1	13.10.2016	12.251	87.749	0.780 ± 0.001	9.370 ± 1.434	0.303± 0.069
Blueray room 2	13.10.2016	11.042	88.958	0.751 ± 0.003	8.430 ± 1.318	0.268± 0.063
Blueray room 3	13.10.2016	11.265	88.735	0.792 ± 0.008	9.120 ± 1.989	0.259± 0.049
Blueray room 1	22.11.2016	13.956	86.044	0.856 ± 0.001	8.688 ± 1.698	0.256± 0.069
Blueray room 2	22.11.2016	13.909	86.091	0.818 ± 0.003	9.040 ± 0.873	0.338 ± 0.075
Blueray room 3	22.11.2016	11.879	88.121	0.705 ± 0.005	10.400 ± 1.268	0.283 ± 0.124

Table 3 shows that the dry matter and water content for Chandler variety had small variations in room 2 and 3 from the initial moment (0) compared with room 1 (control). For control room (CO_2 - 0%) it have noted more fluctuations. Titratable acidity (TA) values were maintained with small variations during storage (Yang et al., 2014).

Table 3.Variation of firmness and content of: dry matter, water, TA and soluble solids during storage period in CA for Chandler variety

Sample	Time of analysis	Dry matter content(D.M. %)	Water content (%)	Titratable acidity (TA)(g acid citric/100 g)	Soluble solids (% Brix)	Firmness (kg/cm^2)
Chandler	17.08.2016	12.693	87.307	0.851± 0.009	7.390±1.480	0.079 ± 0.047
Chandler room 1	13.10.2016	13.297	86.703	0.836 ± 0.002	10.050±1.706	0.322 ± 0.037
Chandler room 2	13.10.2016	11.604	88.396	0.909 ± 0.002	10.370±1.113	0.272 ± 0.049
Chandler room 3	13.10.2016	12.413	87.587	0.889 ± 0.005	10.170±1.501	0.274 ± 0.064
Chandler room 1	22.11.2016	13.381	86.619	0.863 ± 0.014	9.560±1.692	0.280 ± 0.035
Chandler room 2	22.11.2016	12.626	87.374	0.833 ± 0.001	10.725±1.320	0.340 ± 0.062
Chandler room 3	22.11.2016	11.352	88.648	0.858 ± 0.003	9.440±1.665	0.315 ± 0.036
Chandler room 1	12.12.2016	11.216	88.784	0.820 ± 0.003	8.750±1.925	0.320 ± 0.049
Chandler room 2	12.12.2016	13.554	86.446	0.857 ± 0.001	9.830±0.953	0.333 ± 0.053
Chandler room 3	12.12.2016	12.686	87.314	0.759 ± 0.001	9.750±0.977	0.278 ± 0.049

A noticeable increase in soluble solids content in all rooms throughout storage can be observed. For the firmness of Chandler variety fruits, an important increase in all rooms throughout the storage period from the initial moment (0) has been observed.

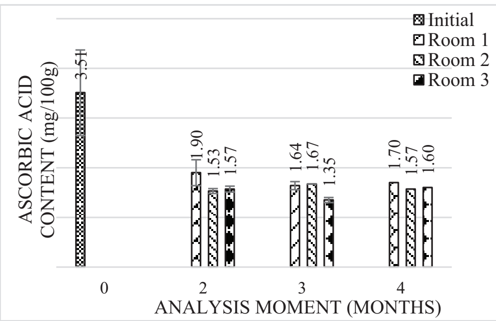


Figure 1. Variation of ascorbic acid content (mg/100g) during storage period in CA for Coville variety where: 0-initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage, 4 - analyses after 4 months of storage

The ascorbic acid content of Coville variety (Figure 1), has declined during storage. Between the three type of storage, there were small differences.

Also at Bluray variety (Figure 2) low values during storage were determined when compared with the initial moment.

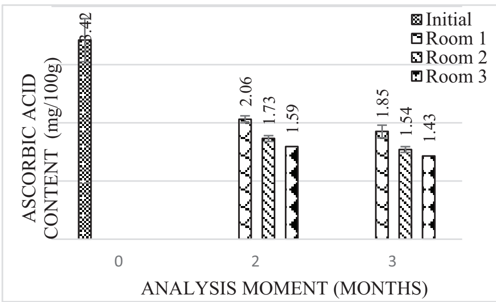


Figure 2. Variation of ascorbic acid content (mg/100g) during storage period in CA for Blueray variety where: 0 - initial moment, 2 – analyses after 2 months of storage, 3- analyses after 3 months of storage

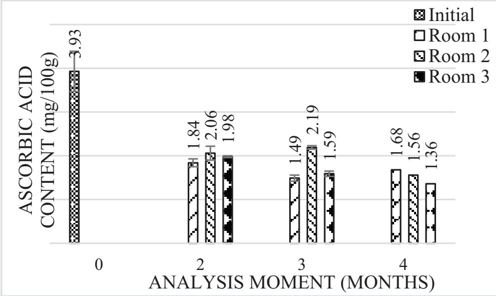


Figure 3. Variation of ascorbic acid content (mg/100g) during storage period in CA for Chandler variety where: 0 - initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage, 4 - analyses after 4 months of storage

In room 1 (CO_2 - 0%) higher values from all the storage variants were present. At Chandler variety (Figure 3) higher values of ascorbic acid content during the storage in the case of room 2 (CO_2 - 5%) were observed.

Koyuncu (2010) showed that the ascorbic acid content of the fruits progressively drops during storage in cold rooms with T: 1 °C and RH: 95%.

Anthocyanins are water-soluble pigments and belong to flavonoid group. They have a very high antioxidant capacity and are responsible for the color in red-purple fruits.

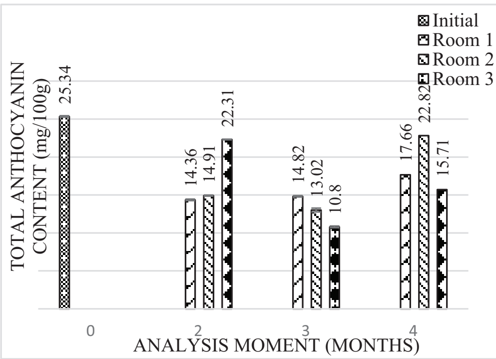


Figure 4. Variation of total anthocyanin content (mg/100g) during storage period in CA for Coville variety where: 0 - initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage, 4 - analyses after 4 months of storage

The fruits content of anthocyanins can also correlate with the antioxidant capacity (Matityahu, 2016).

Coville variety had the best results regarding total anthocyanins content after four months of storage in the room 2 (CO2 - 5%), while for the fruits stored two months the decrease in anthocyanins was at half comparative to the initial moment (Figure 4).

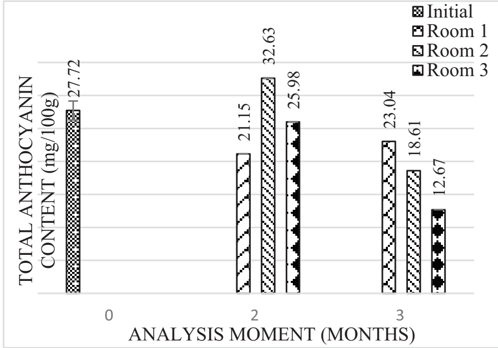


Figure 5. Variation of total anthocyanin content (mg/100g) during storage period in CA for Blueray variety where: 0 - initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage

After two months of storage in controlled atmosphere from room 2, the total anthocyanins content for Blueray variety registered an increase with 17% compared to the initial moment. After three months of storage, total anthocyanins content value was lower compared to the registered value from the initial moment (Figure 5).

At Chandler variety (Figure 6), the total anthocyanins content increased during storage in room 1 (CO_2 - 0%) having the highest value after two months of storage.

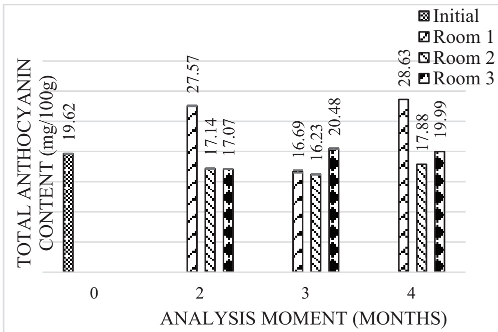


Figure 6. Variation of total anthocyanin content (mg/100g) during storage period in CA for Chandler variety where: 0 - initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage, 4 - analyses after 4 months of storage

The values of anthocyanins content of the fruits from room 3 were comparable to the ones from the initial moment.

Higher values of the total anthocyanins content towards initial moment, indicate that at low temperatures post-ripening process of fruits continues (Matityahu, 2016). The fruits from rooms with CO_2 had total anthocyanins values content close to the initial moment.

The content of total polyphenols in the blueberries of the 3 varieties studied, increased gradually towards the initial moment throughout storage. At the Coville variety (Figure 7) lower values were observed at the end of the storage in room 3 (CO_2 - 10%) compared to other rooms.

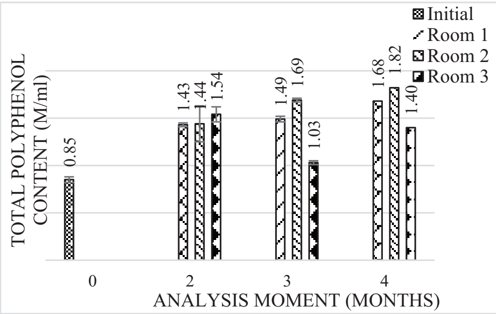


Figure 7. Variation of total polyphenol content during storage period in CA for Coville variety where:
0 - initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage, 4 - analyses after 4 months of storage

Blueray variety (Figure 8) recorded a lower total polyphenol content in rooms 2 and 3 (with CO_2) compared with the blueberries from room 1 (without CO_2) after 2 and 3 months of storage.

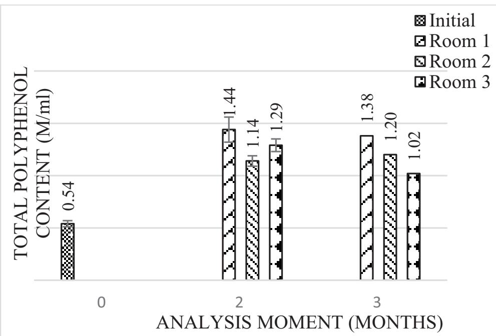


Figure 8. Variation of total polyphenol content during storage periodin CA for Blueray variety where: 0 - initial moment, 2 - analyses after 2 months of storage,
3 - analyses after 3 months of storage

For Chandler variety (Figure 9) in room 1 (without CO_2) there was a progressive increase of total polyphenol content. Yang G. (2014) suggests that this increase in polyphenol content during storage is a process of maturing fruits which were picked when they were not at fully maturity for consumption.

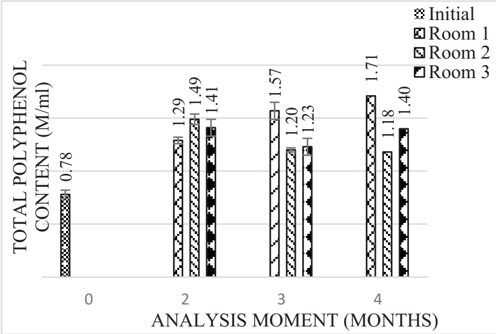


Figure 9. Variation of total polyphenol content during storage period in CA for Chandler variety where:
0 -initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage, 4 - analyses after 4 months of storage

The total flavonoid content of Coville variety (Figure 10) has recorded lower values for all three rooms in 2, 3 and 4 months compared with the value from the initial moment.

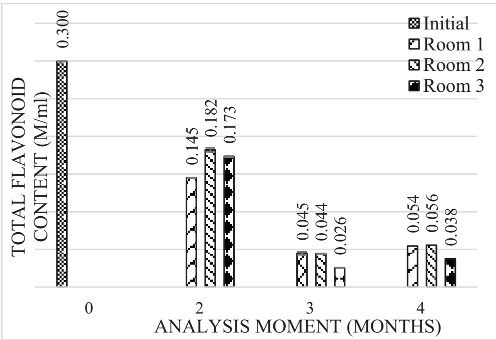


Figure 10. Variation of total flavonoid content during storage period in CA for Coville variety where: 0 - initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage, 4 - analyses after 4 months of storage

The highest content in flavonoids for Blueray variety (Figure 11) was noticed in room 1 (without CO_2) after two months of storage.

The Chandler variety (Figure 12) maintained the flavonoids content after two months of storage in room 1 (CO2 - 0%) and 2 (CO2 - 5%), and in room 3 (CO2 - 10%) a small decrease of the values was recorded.

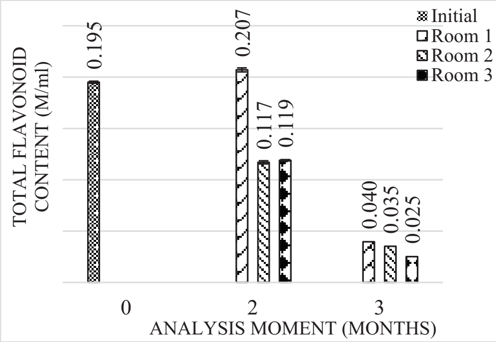


Figure 11. Variation of total flavonoid content during storage period in CA for Blueray variety where: 0 - initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage

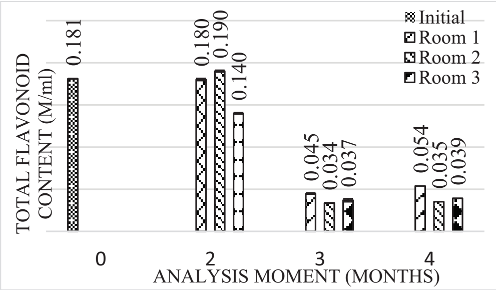


Figure 12. Variation of total flavonoid content during storage period in CA for Chandler variety where: 0 -initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage, 4 - analyses after 4 months of storage

All varieties studied showed an important antioxidant capacity. Coville variety (Figure 13) recorded lower values for antioxidant activity in room 1 (CO_2 - 0%) and room 2 (CO_2 - 5%) after 2 months of storage, compared to the value of the initial moment.

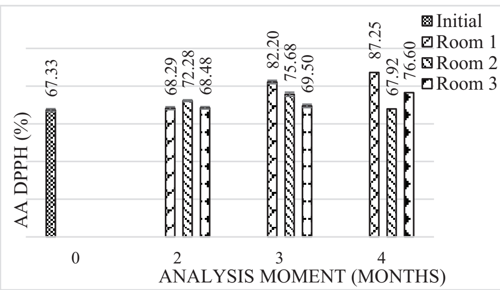


Figure 13. Variation of AA DPPH(%) content during storage period in CA for Coville variety where: 0 - initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage, 4 - analyses after 4 months of storage

Blueray variety (Figure 14) recorded lower values for the antioxidant activity in all three rooms at 2 and 3 months compared to the value of the initial moment.

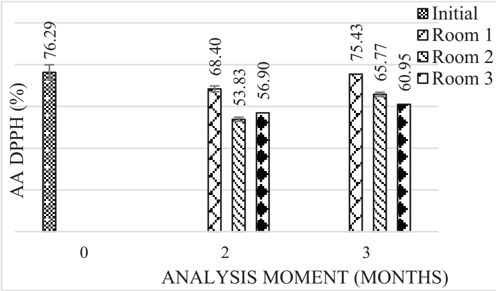


Figure 14. Variation of AA DPPH(%) content during storage period in CA for Blueray variety where: 0 - initial moment, 2 - analyses after 2 months of storage, 3 - analyses after 3 months of storage

Chandler variety (Figure 15) had the strongest antioxidant activity after four months of storage in the room 1 (without CO_2).

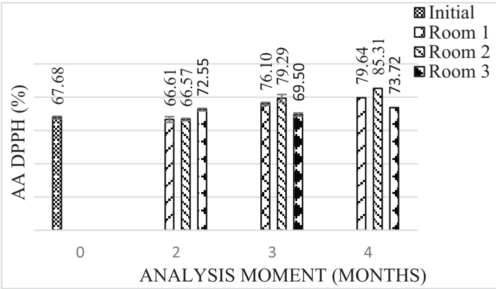


Figure 15. Variation of AA DPPH(%) content during storage period in CA for Chandler variety where: 0 -initial moment, 2 - analyses after 2 months of storage, 3analyses after 3 months of storage, 4 - analyses after 4 months of storage

CONCLUSIONS

The storage in rooms with controlled atmosphere influenced, as expected, fruit quality parameters. It can be noted that all varieties of samples did not have the same level of maturity at harvest, Coville and Blueray varieties being collected at the end of the harvest, while Chandler variety was collected midterm harvest. Because of that, Chandler variety behaved much better during the four months of storage compared to the other two varieties, maintaining much better visual, organoleptic and economical properties.

In the case of antioxidant capacity of Coville and Chandler it was observed that room 3 (CO_2 - 10%) had a slight increase towards the end of the storage period suggesting that metabolic processes in fruit were slowed down due to higher CO_2 content.

The varieties behaved differently, observing for example the content of ascorbic acid. The Coville and Blueray varietes behaved better in the room without CO_2 while for the variety Chandler observations concluded that it maintained a higher quantity of ascorbic acid in room 2 (CO_2 - 5%).

Following the obtained results, we can specify that the varieties of the same species (blueberry - *Vaccinium corymbosum* L.) requires different storage conditions depending on crop technology applied and harvesting moment.

The post ripening continued in all rooms but was slowed down in rooms 2 and 3 (with CO_2) compared to room 1 (without CO_2). Small differences were observed in flavonoids and ascorbic acid content of the blueberries between the two rooms with CO_2 (room 2: 5% and room 3: 10%).

REFERENCES

Bărăscu R., Hoza D., Bezdadea-Cătuneanu I., Năftănăilă M., Albulescu A., 2016. Preliminary research regarding the grafting interstock and soil maintenance influence on fruit quality for Pinova Variety. Agriculture and Agricultural Science Procedia 10: 167-171.

Bessemans N., Verboven P., Verlinden B.E., Nicolaï B.M., 2016. A novel type of dynamic controlled atmosphere storage based on the respiratory quotient (RQ-DCA). Postharvest Biology and Technology 115: 91-102

Chen H., Cao S., Fang X., Mu H., Yang H., Wang X., Xu Q.,Gao H.,2015. Changes in fruit firmness, cell wall composition and cell wall degrading enzymes in postharvest blueberries during storage. ScientiaHorticulturae188: 44-48.

Corollaro M. L., Aprea E., Endrizzi I., Betta E., Demattè M. L., Charles M., Bergamaschi M., Costa F., Biasioli F., Grappadelli L. C., Gasperi F., 2014. A combined sensory-instrumental tool for apple quality evaluation. Postharvest Biology and Technology 96: 135-144

Cortellino G., Piazza L., Spinelli L., Torricelli A., Rizzolo A., 2017. Influence of maturity degree, modified atmosphere and anti-browning dipping on texture changes kinetics of fresh-cut apples. Postharvest Biology and Technology 124: 137-146

DeEll J., Prange R., 1992. Postharvest quality and sensory attributes of organically and conventionally grown apples. HortScience 27(10): 1096-1099.

Delian E., Petre V., Burzo I., Bădulescu L., Hoza D., 2011. Total phenols and nutrients composition aspects of some apple cultivars and new studied breeding creations lines grown in Voineşti area – Romania. Romanian Biotechnological Letters vol. 16, no. 6

Drogoudi P., Pantelidis G., Goulas V., Manganaris G., Ziogas V., Manganaris A., 2016. The appraisal of qualitative parameters and antioxidant contents during postharvest peach fruit ripening underlines the genotype significance. Postharvest Biology and Technology 115: 142-150

Francini A., Sebastiani L., 2013. Phenolic Compounds in Apple (*Malus domestica* Borkh.): Compounds Characterization and Stability during Postharvest and after Processing. Antioxidants, 2(3), 181–193

Khanizadeh S., Tsao R., Rekika D., Yang R.,Charles M., Rupasinghe V., 2008. Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. Journal of Food Composition and Analysis 21: 396-401

Koyuncu M., Dilmaçünal T., 2010. Determination of Vitamin C and Organic Acid Changes in Strawberry by HPLC During Cold Sorage. Notulae Botanicae Horti Agrobotanici, 38(3): 95-98

Li D., Li B., Ma Y., Sun X., Lin Y., Meng X., 2017. Polyphenols, anthocyanins, and flavonoids contents and the antioxidant capacity of various cultivars of highbush and half-high blueberries. Journal of Food Composition and Analysis, ISSN 0889-1575, http://dx.doi.org/10.1016/j.jfca.2017.03.006.

Liato V., Hammami R., Aïder M., 2017. Influence of electro-activated solutions of weak organic acid salts on microbial quality and overall appearance of blueberries during storage. Food Microbiology, vol 64: 56-64

Matityahu I., Marciano P., Holland D., Ben-Arie R., Amir R., 2016. Differential effects of regular and controlled atmosphere storage on the quality of three cultivars of pomegranate (*Punica granatum* L.). Postharvest Biology and Technology 115: 132-141

Moura C., Masson M., Yamamoto C., 2005. Effect of osmotic dehydration in the apple (*Pyrus malus*) varieties Gala, Gold and Fuji. Thermal Engineering, vol 4: 46-49

Mureşan E., Muste S., Borşa A., Vlaic R., Mureşan V., 2014. Evalution of physical-chemical indexes, sugars, pigments and phenolic compounds of fruits from three apple varieties at the end of storage period. Bulletin UASVM Food Science and Technology 71(1)

Oltenacu N., Lascăr E., 2015. Capacity of maintaining the apples quality, in fresh condition-case study. Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development vol. 15: 331-335

Rizzolo A., Vanoli M., Spinelli L., Torricelli A., 2010. Sensory characteristics, quality and optical properties measured by time-resolved reflectance spectroscopy in stored apples. Postharvest Biology and Technology 58: 1-12

Saei A., Tustin D., Zamani Z., Talaie A., Hall A., 2011. Cropping effects on the loss of apple fruit firmness during storage: The relationship between texture retention and fruit dry matter concentration. Scientia Horticulturae 130: 256-265

Shen Y., Zhang H., Cheng L., Wang L., Qian H., Qi X., 2016. In vitro and *in vivo* antioxidant activity of polyphenols extracted from black highland barley. Food Chemistry 194: 1003–1012

Shi M., Loftus H., McAinch A.J., Su X.Q., 2017. Blueberry as a source of bioactive compunds for the treatment of obesity, type 2 diabetes and chronic inflammation. Journal of Functional Foods 30: 16-29

Skupień K., 2006. Chemical composition of selected cultivars of highbush blueberry fruit (*Vaccinium corymbosum* L.). Folia Horticulturae 18/2: 47-56

Song Y., Huang L., Yu J., 2016. Effects of blueberry anthocyanins on retinal oxidative stress and inflammation in diabetes through Nrf/HO-1 signaling. Journal of Neuroimmunology 301: 1-6

Ticha A., Salejda A., Hyšpler R., Matejicek A., Paprstein F., Zadak Z., 2015. Sugar composition of apple cultivars and its relationship to sensory evaluation. Nauka. Technologia. Jakość 4(101): 137-150

Varela P., Salvador A., Fiszman S., 2008. Shelf-life estimation of "Fuji" apples II. The behaviour of recently harvested fruit during storage at ambient conditions. Postharvest Biology and Technology 50: 64-69

Wang H., Guo X., Hu X., Li T., Fu X., Liu R.H., 2017. Comparison of phytochemical profiles, antioxidant and cellular antioxidant activities of different varieties of blueberry (*Vaccinium* spp.). Food Chemistry 217: 773-781

Xu F., Wang S., Xu J., Liu S., Li G., 2016. Effects of combined aqueous chlorine dioxide and UV-C on shelf-life quality of blueberries. Postharvest Biology and Technology, 117: 125-131

Yang G., Yue J., Gong X., Qian B., Wang H., Deng Y., 2014. Blueberry leaf extracts incorporated chitosan coatings for preserving postharvest quality of fresh blueberries. Postharvest Biology and Technology, 92: 46-53

Yoon K. Y., Woodams E. E., Hang Y.D., 2005. Relationship of acid phosphatase activity and Brix/acid ratio in apples. Lebensm.-Wiss. u.-Technol, 38: 181-183

Žilić S., Šukalović V. H. T., Dodig D., Maksimović V., Maksimović M., BasićZorica, 2011. Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. Journal of Cereal Science 54: 417-424.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF AMELIORATIVE PLANTS AND MULCH ON SOME SOIL AGROCHEMICAL CHARACTERISTICS IN AN ORGANIC EDIBLE ROSE CROP

Ana Cornelia BUTCARU, Florin STĂNICĂ, Roxana MADJAR

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd., 011464, Bucharest, Romania

Corresponding author email: anabutcaru@gmail.com

Abstract

The paper presents the evolution of some soil agrochemical parameters: pH, mineral N, P_{AL}, K_{AL} and humus, between March 2015 and November 2016, in an organic edible rose culture under the influence of three ameliorative species and two mulching systems. With the goal of planting three edible rose varieties, in the experimental field of USAMV Bucharest, a special soil preparation was applied in the spring of 2015. Three ameliorative plants, Sinapis alba L., Tagetes patula L. and Phacelia tanacetifolia L., with role in soil disinfection were used in seven different combinations (V1-V7) and a control plot was kept without seeding (V8). After flowering and seed formation, the mature plants were trimmed and incorporated into the soil. After the organic roses planting, the same variants were seeded between the rose rows in the spring of 2016. In the summer of 2016, two mulching variants were applied for each initial variant (Vn), on the roses rows: Vn.1. wood chips and Vn.2. wool, while the control Vn.3., was represented by unmulched soil. The results show important changes on soil characteristics due to the influence of ameliorative species and mulch systems. All the wool variants (Vn.2.) have an important increase of N mineral, from an initial average of 3.375 ppm to 51.375 ppm at V1.2. Sinapis compared to 23.125 at V8.2.Control. The P content increased from 192 ppm to 398 ppm in V4.3. - Sinapis + Tagetes variant. The K content increased from 274.56 ppm in the initial stage to a maximum of 800 ppm in the V1.2. - Sinapis variant on wool mulched row. The humus content modified from 2.37% to 3.12% in more variants (V1.2., V2.3., V2.2., V6.1.). V1.2. Sinapis with wool mulch variant presents the best improvement of agrochemical parameters (pH 7.09; mineral N 51.375 ppm; K 800 ppm and humus 3.12%) compared with the others variants.

Key words: *pH, NPK, humus, wood chips, wool.*

INTRODUCTION

The first principle of organic agriculture, Health, established by IFOAM (IFOAM, 2010) sustain that health of soil, plant, animal, human and planet to be viewed as one and indivisible.
Cover crops and living mulch can be an important component of increasing the fertility and health of soil (Crossland et al., 2015).
Different kind of organic matter have additional positive effects on yield through amelioration of soil life, water retention, humus content and other aspects (van Opheusden et al., 2012; Butcaru et al., 2016; Butcaru et al., 2015).
Intercropping can be a way of increasing crop diversity, especially in the perennial culture (Andersen, 2005; Butcaru et al., 2016).
In the same time, as it is stated by principle of ecology (IFOAM, 2010), the organic agriculture should be based on living ecologicalsystems and cycles, work with them, emulate them and help sustain them.
One of the most important aspects in organic agriculture is improving and maintaining soil organic matter (Reeve, 2007; Berca, 2011).
The availability of nutrients is an important factor in plant growth and was investigated in different organic substrates (Madjar et al., 2004).
The present paper presents the results after using an alternative and innovative method for improving the soil activity by using three ameliorative species: *Sinapis alba* L., *Tagetes patula* L. Sparky Mix and *Phacelia tanacetifolia* L., before and after the plantation of an organic edible rose culture.
From the first year of plantation, two kind of mulch was used: wood chips and wool.
The soil parameters (pH, mineral N, P_{AL}, K_{AL}, humus content), measured before the establishment of the edible rose culture and after one year, reflect the potential of the ameliorative plants and in the same time of the mulch as fertilizer.

MATERIALS AND METHODS

With the special goal of planting three edible rose varieties using an organic technology, an experimental plot at the University of Agronomic Sciences and Veterinary Medicine of Bucharest of a total area of 1,350 m^2 was used.

A special soil preparation was applied beginning with the spring of 2015. Three ameliorative plants, *Sinapis alba* L., *Tagetes patula* L. and *Phacelia tanacetifolia* L., with rolein soil disinfection were used.

Sinapis alba L. (Fam. *Brassicaceae)* is an important spicy and honey plant withmedicinal properties, widely usedas agreen manure and also as an ameliorative plant, effective in fighting soil erosion (De Baets et al., 2011) and weeding etc.

Phacelia tanacetifolia L.(Fam. *Boraginaceae*) has a strong nematocide action; it fixes nitrates in the roots; eliminates weeds, being used to control couch grass (Berca, 2011); presents allopathic effects (Dhimaet et al., 2010). It is a very good honey plant. Liuet et al., 2013, showed the high potential for both cultures to take up phosphorus from the soil and release it later. *Tagetes patula* L. Sparky Mix (Fam. *Asteraceae)* is an ornamental and medicinal plant, used in crop protection due to its natural content of fungicides, insecticides and nematocides substances; controls many nematode species (Hookset et al., 2010).

Crops were sown in late March, by combining the three species in 7 variants: V1 *Sinapis*, V2 *Sinapis* + *Phacelia*, V3 *Phacelia*, V4 *Sinapis* + *Tagetes*, V5 *Sinapis* + *Tagetes* + *Phacelia*, V6 *Tagetes* + *Phacelia*, V7 *Tagetes* and a control parcel V8, was kept as black field, without sowing. After plugging and soil preparation with a rotary cutter, sowing was done simultaneously for all three cultures, with 19kg seeds/ha for *Sinapis*, 38.5 kg seeds/ha for *Phacelia* and 7.5 kg seeds/ha for *Tagetes*.

Sinapis alba L. and *Phacelia tanacetifolia* L. were cultivated without irrigation in the period March to June 2015, the date on which they were trimmed and incorporated into the soil. *Tagetes patula* L. Sparky Mix was irrigated from June to September 2015, when it was incorporated into the soil.

After the organic roses planting, the same variants were seeded between the rose rows in the spring of 2016 and the mature plants were trimmed and incorporated into the soil, all three species in the same time in June 2016.

The roses, planted on three rows on each variant (V1-V8), were supported by wire trellis. A drip system, beginning with July 2016, was installed and operational.

In the summer of 2016, two mulching variants were applied for each initial variant (Vn), on the roses rows: Vn.1. wood chips and Vn.2. wool, while the control Vn.3., was represented by unmulched soil. Both mulched rows had the same 1 m width with the specific material.

Wool is an organic compound recommended also as fertilizer, with 5-6% N, 2-4% P, 1-3% K with a range of effectiveness of 4-9 months after applying (Penhallegon, 2003).

Wood chips are widely recommended in horticulture as mulch.

The inter-row was kept grassy through repeated mowing.

For each variant (V1-V8) was applied the same scheme of treatment, including: fertilising with manure in autumn 2015 at planting and organic products in 2016; plant protection with different organic products; bio stimulatory and caw milk for increasing the immunity system.

For the analysis of the agrochemical characteristics, soil samples were collected from the total area in March 2015, from each variant (Vn.) in October 2015 and from each subvariant (Vn.1. Vn.2., Vn.3.) in November 2016. Agrochemicalanalysisdetermined themineral N, mobile forms of P_{AL} and K_{AL}, the amount of humus and soil pH on two horizons 0-20 cm and 20-40 cm. Measurements were carried out according to the following methodologies: soil moisture by gravimetric method, pH by potentiometric method in aqueous suspension (1:2.5),mineral nitrogen as sum of ammonium and nitrate available in soil evaluated by spectrophotometry, mobile forms of $P_{AL}$and $K_{AL}$by Egner - Riehm - Domingo method, humus content was calculated from organic carbon determination with Walkley - Black - Gogoașă method.

RESULTS AND DISCUSSIONS

The results show important improvement in the soil parameters, specific on each variant.

Table 1. Evolution of pH parameter for 0-20 cm horizon between March (2015) and November (2016) period

Variant	Mar. 2015	Oct. 2015	Nov. 2016		
			Vn.1. Wood chips	Vn.2. wool	Vn.3. un-mulched
V1 – *Sinapis*	7.33	7.58	7.56	7.09	7.60
V2 – *Sinapis* + *Phacelia*		7.27	7.58	7.24	7.61
V3 – *Phacelia*		7.68	7.59	7.54	7.67
V4 – *Sinapis* + *Tagetes*		7.78	7.09	7.07	7.05
V5 – *Sinapis* + *Tagetes* + *Phacelia*		7.68	7.14	7.06	7.24
V6 – *Tagetes* + *Phacelia*		7.93	7.44	7.26	7.48
V7 – *Tagetes*		8.01	7.60	7.31	7.56
V8 – Control		7.90	7.61	7.38	7.55

After an increase of the pH value during the 2015 year for all variants (V1-V8) from a very slightly alkaline to slightly alkaline, at the end of the 2016 year the pH attended values in the scale of neutral reaction for V1.2.-*Sinapis* with wool mulch, V4 –*Sinapis* + *Tagetes* in all three sub-variants (V4.1., V4.2., V4.3.), V5.1.-*Sinapis* + *Tagetes* + *Phacelia* with wood chips and wool.

The sub-variants Vn.2. mulched with wool presents the bigger decreases towards the wood chips mulched and un-mulched sub-variants (Figure 1).

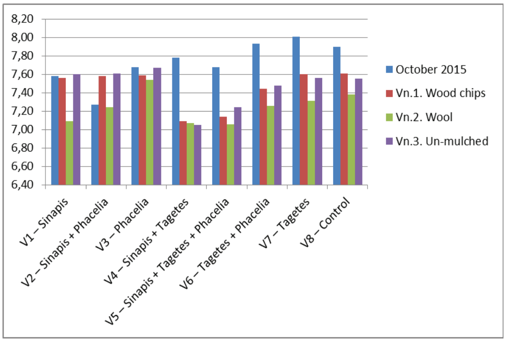


Figure 1. Influence of ameliorative species and mulch on the evolution of pH parameter

In the 0-20 cm horizon, an important evolution was made by the mineral nitrogen, mobile forms of phosphorus and potassium and by humus content.

Table 2. Evolution of mineral N (ppm) for 0-20 cm horizon between March (2015) and November (2016) period

Variant	Mar. 2015	Oct. 2015	Nov. 2016		
			Vn.1. Wood chips	Vn.2. Wool	Vn.3. Un-mulched
V1 – *Sinapis*	3.125	3.625	3.250	51.375	4.500
V2 – *Sinapis* + *Phacelia*		15.875	3.375	41.125	5.250
V3 – *Phacelia*		1.625	13.875	39.250	16.000
V4 – *Sinapis* + *Tagetes*		3.250	3.250	47.250	10.500
V5 – *Sinapis* + *Tagetes* + *Phacelia*		3.500	3.500	40.750	12.750
V6 – *Tagetes* + *Phacelia*		2.500	10.000	53.000	19.375
V7 – *Tagetes*		4.750	3.000	25.375	7.000
V8 – Control		7.375	8.875	23.125	16.125

The bigger mineral N value increases were at all Vn.2. sub-variants, from 1.625 ppm to 39.250 ppm (V3.2. *Phacelia*), 2,500 ppm to 53.000 ppm (V6.2.*Tagetes* + *Phacelia*) or 3.625 ppm to 51, 375 ppm (V1.2.*Sinapis*).

The smallest value at Vn.2. was in the V8 - control variant.

In the V3 - *Phacelia* variant were important increase in all three sub-variants with mulch or un-mulched (Figure 2).

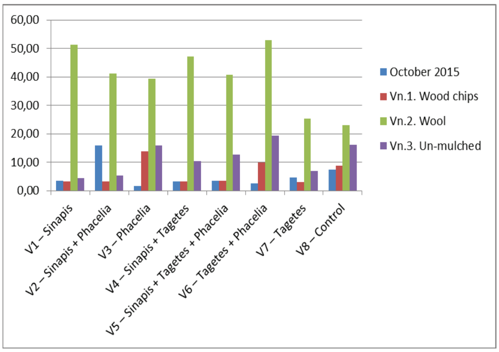


Figure 2. Influence of ameliorative species and mulch on the evolution of mineral N (ppm) parameter

In the wood chips sub-variants, some of them showed a small increase of mineral N (V3.1., V6.1., V8.1.) but most of them presented not influence or decrease of mineral N.

The un-mulched sub-variants present increases of mineral N due to the incorporating the mowed weeds.

Table 3. Evolution of P (ppm) for 0 - 20 cm horizon between March (2015) and November (2016) period

Variant	Mar. 2015	Oct. 2015	Nov. 2016		
			Vn.1. Wood chips	Vn.2. Wool	Vn.3. Un-mulched
V1 – *Sinapis*	192.00	204.40	215.20	281.20	270.40
V2 – *Sinapis* + *Phacelia*		158.80	280.80	242.00	293.20
V3 – *Phacelia*		189.20	174.40	222.40	244.80
V4 – *Sinapis* + *Tagetes*		457.20	329.60	326.00	398.00
V5 – *Sinapis* + *Tagetes* + *Phacelia*		200.00	216.80	222.80	200.40
V6 – *Tagetes* + *Phacelia*		186.40	216.00	238.80	243.20
V7 – *Tagetes*		163.30	192.80	268.80	216.20
V8 – Control		107.60	184.40	181.60	202.40

The P content increases in general in the second year of the research, being at a very high level on the scale.

The V4 variant present a decrease from the October 2015 value, but all the values are bigger than the initial one (March 2015).

The influences of wool much were bigger that of wood chips in the variation of P content. The un-mulched variants presents on average bigger increases than the mulched rows (Figure 3).

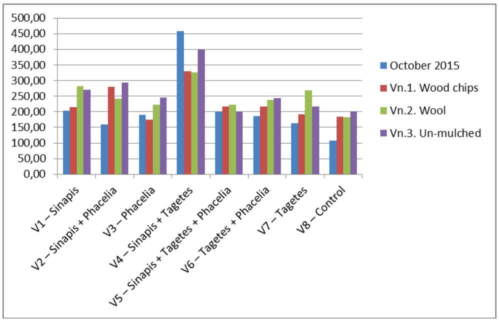


Figure 3. Influence of ameliorative species and mulch on the evolution of P (ppm) parameter

In general, all the variants influenced more the increase of P content than the V8 - control plot.

Table 4. Evolution of K (ppm) for 0 - 20 cm horizon between March (2015) and November (2016) period

Variant	Mar. 2015	Oct. 2015	Nov. 2016		
			Vn.1. Wood chips	Vn.2. Wool	Vn.3. Un-mulched
V1 – *Sinapis*	274.65	349.30	400.00	800.00	420.00
V2 – *Sinapis* + *Phacelia*		287.56	426.00	720.00	440.00
V3 – *Phacelia*		301.93	280.00	560.00	426.00
V4 – *Sinapis* + *Tagetes*		316.31	420.00	600.00	400.00
V5 – *Sinapis* + *Tagetes* + *Phacelia*		388.20	402.00	520.00	410.00
V6 – *Tagetes* + *Phacelia*		474.47	404.00	640.00	430.00
V7 – *Tagetes*		316.31	320.00	524.00	390.00
V8 – Control		316.31	280.00	390.00	320.00

The potassium content increases in general in all variants and sub-variants to a very high content on the scale.

The most important ones are in the Vn.2. sub-variants were are practically more than doubled the amount of potassium (V1.2., V2.2.).

The control plot (V8) had the smallest increase from 316,31 to 390, 00 ppm (Figure 4).

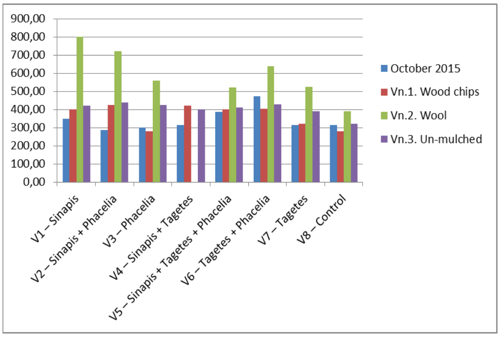


Figure 4. Influence of ameliorative species and mulch on the evolution of K (ppm) parameter

The wood chips and un-mulched sub-variants presented closed values of potassium content.

Table 5. Evolution of humus content (%) for 0-20 cm horizon between March (2015) and November (2016) period

Variant	Mar. 2015	Oct. 2015	Nov. 2016		
			Vn.1. Wood chips	Vn.2. Wool	Vn.3. Un-mulched
V1 – *Sinapis*	2.37	2.49	2.99	3.12	2.87
V2 – *Sinapis* + *Phacelia*		2.24	2.87	3.12	3.12
V3 – *Phacelia*		1.87	2.24	2.99	2.24
V4 – *Sinapis* + *Tagetes*		2.12	2.87	2.49	2.99
V5 – *Sinapis* + *Tagetes* + *Phacelia*		2.37	2.87	2.62	2.62
V6 – *Tagetes* + *Phacelia*		2.74	3.12	2.62	2.87
V7 – *Tagetes*		2.80	2.62	2.87	2.74
V8 – Control		2.49	2.74	2.24	2,24

The humus content increased, being at a medium range on the scale.

The mulched rows influenced in average the same the increase of humus content, more than the un-mulched row (Figure 5).

The control plot has the smallest increases in general comparative with the other variants.

Ameliorative plants used: *Sinapis alba*, *Phacelia tanacetifolia*, *Tagetes patula* \`Sparky mix\` proved good qualities as ameliorative plants combined also with the two mulch systems – wood chips and wool.

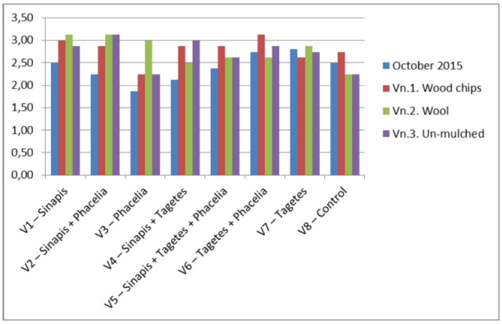


Figure 5. Influence of ameliorative species and mulch on the evolution of humus content (%)

Humus content (%) increased from the initial stage and maintained the positive evolution, as noted also by Crossland et al. (2015), van Opheusden et al. (2012); Butcaru et al. (2016), Reeve (2007), Berca (2011).

Using the ameliorative plants between the rose rows increased crop diversity with positive effects on the basic culture (Andersen, 2005; Butcaru et al., 2016).

Wool, used as mulch, proved also its capacity as fertilizer. Notable increases in Nmineral, P and especially on K in four months after applying recommended it as a good material in orchards as noted also by Penhallegon (2003).

CONCLUSIONS

All three ameliorative plants *Sinapis alba, Phacelia tanacetifolia* and *Tagetes patula* proved important qualities regarding the improvement of the soil parameters (pH, mineral N, mobile forms of P and K, humus content).

Wood chips and wool used as two mulched variants showed important increases in mineral N, K and humus content, especially the second, demonstrating the fertilising quality as well.

In the same time, the pH decreased to a neutral and very slightly alkaline reaction.

REFERENCES

Andersen M. K., 2005.Competition and complementarity in annual intercrops – the role of plant available nutrients. Department of Agricultural Sciences Environment, Resources and Technology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark, p. 3

Berca M., 2011. Agrotehnică – Transformarea modernă a agriculturii. Ed. Ceres, București, p.173.

Butcaru A.C., Stănică F., Matei G. M., Matei S., 2016. Alternative methods to improve soil activity before planting an organic edible rose crop, Journal of Horticulture, Forestry and Biotechnology, Volume 20(4), p. 12-17, ISSN: 2066-1797

Butcaru A.C., Stănică F., Matei G.M., Matei S., 2015. Pregătirea solului în vederea înființării unei culturi de trandafiri de dulceață în sistem ecologic, revista Hortus nr.14, p. 165-168

Crossland M., Fradgley N., Creissen H., Howlett S., Baresel P., Finckh M. and Girling R, 2015. An online toolbox for cover crops and living mulches, Aspects of Applied Biology - Getting the Most out of Cover Crops, Volume 129, p. 1

De Baets S., Poesen J., Meersmans J., Serlet L., 2011.Cover crops and their erosion-reducing effects during concentrated flow erosion. Catena Journal 85, ISSN 0341-8162 DOI: 10.1016/j.catena. 2011.01.009, p. 237–244.

Dhima K., Vasilakoglou I., Garane V., Ritzoulis C., VaiaLianopoulou, Eleni Panou-Philotheou, 2010. Competitiveness and Essential Oil Phytotoxicity of Seven Annual Aromatic Plants. Weed Science 58,ISSN1550-2759 DOI:10.1614/WS-D-10-00031.1, p.457–465.

Hooksa C.R.R., Wangb K., Ploegc A., Mcsorleyd R, 2010.Using marigold (*Tagetes* spp.) as a cover crop to protect crops from plant-parasitic nematodes.Applied Soil Ecology 46, ISSN 0929-1393, p. 307–320.

IFOAM EU GROUP, 2010. Organic food and farming.A system approach to meet the sustainability challenge.

Liu J., Khalaf R., Ulén B., Bergkvist G., 2013. Potential phosphorus release from catch crop shoots and roots after freezing-thawing. Plant Soil Journal, electronic ISSN 1573-5036 DOI 10.1007/s11104-013-1716-y.

Madjar, R., Davidescu, V., 2004. Retention and Migration Process of Potassium in an Organic Substrate for Horticulture. *Acta Hort*. 633:309–314.

Penhallegon R., 2003. Nitrogen-phosphorus-potassium values of organic fertilizers. OSU Extension Service - Lane County Office, Karen Ailor, http://extension.oregonstate.edu/lane/sites/default/files / documents/lc437organicfertilizersvaluesrev.pdf, p. 4

Reeve J. R., 2007.Soil quality, microbial community structure, and organic nitrogen uptake in organic and conventional farming systems. Washington State university; Department of Crop and Soil Sciences; http://www.researchgate.net/publication/228542927

Van Opheusden A.H.M., van der Burgt G.J.H.M., Rietberg P.I.,2012.Decomposition rate oforganic fertilizers: effect on yield, nitrogen availability and nitrogen stock in the soil, LouisBolk Institute, www.louisbolk.org, p. 33

www.ifoam-eu.org/workareas/policy/php/CAP.php, p.7-8

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOIL MICROBIAL ACTIVITY IN AN ORGANIC EDIBLE ROSE CROP

Ana Cornelia BUTCARU[1], Florin STĂNICĂ[1], Gabi-Mirela MATEI[2], Sorin MATEI[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd., 011464, Bucharest, Romania
[2]National Research-Development Institute for Soil Science, Agrochemistry and Environment - ICPA,
61 Mărăști Blvd., 011464, Bucharest, Romania

Corresponding author email: anabutcaru@gmail.com

Abstract

The paper presents the evolution of the microbial activity analyzed through the evolution of the soil respiration, bacteria and fungi density between March 2015 and November 2016, in an organic edible rose culture under the influence of three ameliorative species and two mulching systems. Beginning with the spring of 2015, with the goal of planting an edible rose culture, in the experimental field of USAMV Bucharest, a special soil preparation was applied using three ameliorative plants, Sinapis alba L., Tagetes patula L. and Phacelia tanacetifolia L. They have a special role to control pathogens in soil and were used in seven different combinations (V1-V7) and a control plot was kept without seeding (V8). After flowering and seed formation, the mature plants were trimmed and incorporated into the soil. They were seeded in the organic roses culture also, same variants between the rose rows in the spring of 2016. In the summer of 2016, two mulching variants were applied for each initial variant (Vn), on the rose's rows: Vn.1. wood chips and Vn.2. wool, while the control Vn.3. was represented by unmulched soil. Microbial activity was stimulated especially in variants with two plant species. The highest potential of soil respiration was characteristic for combinations including Tagetes but also in the variant with Sinapis alone that stimulated the bacterial activity in microbial communities. Generally, the bacteria and fungi density and species number was higher in V1-V7 variants than in the V8 control variant. Microbial species identified included ubiquitous bacteria and fungi with high metabolic capabilities to degrade various substrates such as cellulose from vegetal wastes or keratine from sheep wool added, due to efficient production of cellulase and keratinolytic protease enzymes (bacteria from genera Bacillus, Xanthomonas, Actinomycetes and fungi from genera Trichoderma, Aspergillus, Penicillium, Cladosporium, Paecilomyces, Myrothecium), many of them contributing to biological control of potential plant pathogens and nematodes in rose cultures.

Key words: *bacteria, fungi, soil respiration, wool mulch, wood chips mulch.*

INTRODUCTION

One of the most important activities in organic agriculture is maintaining and enhancing the soil health respectively the soil organic matter (IFOAM, 2010; Berca 2011; Reeve, 2007).
In the same time, as it is stated by the principle of ecology (IFOAM, 2010), the organic agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them.
An important component for increasing the soil fertility and health can be green manure, cover crops, living mulch (Crossland et al., 2015).
Different kind of organic matter can bring additional positive effects on yield through amelioration of soil life, water retention, humus content (van Opheusden et al., 2012; Butcaru et al., 2016).
Maintaining diversity is another important aspect for perennial cultures in organic agriculture is. Intercropping can be a way of increasing crop diversity (Andersen, 2005; Butcaru et al., 2016).
The present paper presents the results of the microbiological activity in the soil after using an alternative and innovative method for improving the soil activity by using three ameliorative species: *Sinapis alba* L., *Tagetes patula* L. Sparky Mix and *Phacelia tanacetifolia* L., before and after the plantation of an organic edible rose culture.
In addition to the three ameliorative species, from the first year of plantation, two kind of mulch was used: wood chips and wool.
The research analyses the evolution of bacteria, fungi population and respiration coefficient, measured before the establishment of the edible rose culture and after one year and reflect the potential of the ameliorative plants and mulch to develop and maintain the soil activity.

MATERIALS AND METHODS

The research was conducted in the experimental plot at the University of Agronomic Sciences and Veterinary Medicine of Bucharest of a total area of 1,350 m^2 with the purpose of planting three edible rose varieties using an organic technology.

Beginning with spring of 2015 a special soil preparation was applied using three ameliorative plants, *Sinapis alba* L., *Tagetes patula* L. and *Phacelia tanacetifolia* L., with role in soil disinfection (Butcaru et al., 2015; Butcaru et al., 2016).

Crops were sown in late March, by combining the three species in 7 variants: V1 *Sinapis*, V2 *Sinapis* + *Phacelia*, V3 *Phacelia*, V4 *Sinapis* + *Tagetes*, V5 *Sinapis* + *Tagetes* + *Phacelia*, V6 *Tagetes* + *Phacelia*, V7 *Tagetes* and a control parcel V8, was kept as black field, without sowing.

The same variants were seeded between the rose rows in the spring of 2016, after the organic roses planting.

After flowering and seed formation, the mature plants were trimmed and incorporated into the soil, all three species in the same time in June 2016.

Beginning with July 2016, the roses, planted on three rows on each variant (V1-V8), were supported by wire trellis and a drip system was installed and operational.

In the summer of 2016, two mulching variants were applied for each initial variant (Vn), on the roses rows: Vn.1. wood chips and Vn.2. wool, while the control Vn.3., was represented by un-mulched soil. Both mulched rows had the same 1 m width with the specific material.

The inter-row was kept grassy through repeated mowing.

In each variant (Vn) was applied the same scheme of treatment, including: fertilizing with manure in autumn 2015 at planting and organic products in 2016; bio stimulatory and caw milk for increasing the immunity system and plant protection with different organic.

For the analysis of the soil microbiological activity, samples were collected before and after planting the organic rose culture (from the total area in March 2015, from each variant Vn in July and October 2015, from each sub-variant Vn.1., Vn.2., Vn.3. in November 2016).

Microbiological analysis studied the number of heterotrophic bacteria determined using dilution plate method - by dispersing soil suspensions on the nutrient agar medium; number of microscopic fungi determined by dispersing soil suspension on PDA medium and soil respiration determined through the substrate induced respiration method according to RS-ISI-14240-1-(2012).

The taxonomical identification were carried out on the basis of the cultural, morphological and / or physiological characteristics in accordance with bacteria Identification Manual (Bergey, 1994) and fungi in agricultural soils (Domsch & Gams, 1972).

It has been used circular chromatograms of soil extracts, with diffusion through absorption on paper Whatmann no. 1, argentic coloring, which generates information on biological quality of the soil due to analytical separation and formation of images whose model of consistency, shape, size, color, texture may indicate the degree of soil health, vitality, fertility, the intensity of biotic activity, soil conditions, the complexity of organic matter and the presence of stable humus.

RESULTS AND DISCUSSIONS

The microbiological analyses proved an increased activity of the soil under the influence of alternative and innovative methods applied.

The bacteria population significantly increased in the March 2015 - Novembre 2016, with a relative stabilisation in the last period.

Soil samples collected in 2016 (phase IV) showed a high density of heterotrophic aerobic bacteria relative to gram of dry soil, which ranged from a minimum of 32 x 10^6 viable cells/g dry soil to V8.2. - Control with wool sub-variant to a maximum of 88 x 10^6 viable cells / g dry soil to the V7.2. - *Tagetes* with wood chips sub-variant (Figure 1).

The application of organic technology by using organic materials (wool, wood chips) for mulching and ameliorative crops alone or combined caused significant increases in the number of heterotrophic aerobic bacteria relative to controls plots in general.

In the fourth phase it is visible the stimulatory effect of *Sinapis* and *Tagetes* on the proliferation of bacteria. The most important values of bacterial density registered under the effect of wool mulch were obtained at variant with *Sinapis*, followed by that of *Tagetes* as ameliorative plants, but generally using wool mulch has led to less numerous bacterial populations than in the rest of variants, especially when were used combinations of two species of ameliorative plants.

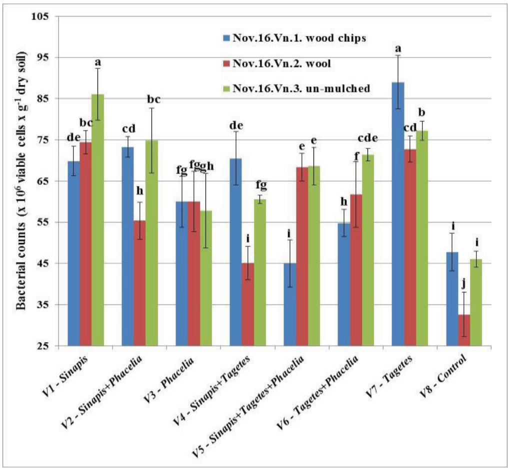


Figure 1. Influence of organic technology on bacterial microflora

Fungal microflora presented moderate values in this stage, below 100 x 10^3 cfu / g of dry soil at control plots and to all variants with wool mulch and values considered high to a number of variants, from which the sub- variants with wood chips V3.1. *Phacelia*, V7.1. *Tagetes* or V1.1. *Sinapis* and the sub-variants un-mulched V7.3. *Tagetes*, V2.3. *Sinapis* + *Phacelia* and V6.3. *Tagetes* + *Phacelia* (Figure 2).

In terms of taxonomy, bacteria and fungi from analyzed soils include ubiquitorius species with high adaptive capacity and species equipped with enzymatic complex equipments, which enable efficient exploitation of a wide variety of substrates with very different origins. There is a considerable number of species capable to degrade and to metabolize organic substrates as wool, wood chips or debris of organic matter due to enzymes such as proteases (keratinases), cellulase and include bacteria belonging to the genera *Bacillus*, *Xanthomonas*, actinomycetes or fungal species from genera *Penicillium*, *Aspergillus*, *Paecilomyces*, *Myrothecium*, *Cladosporium*, *Trichoderma*.

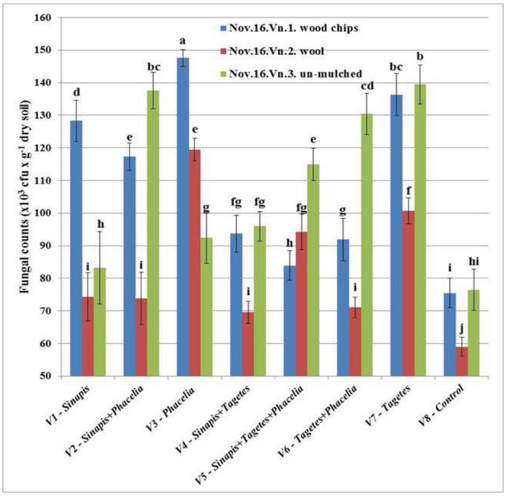


Figure 2. Influence of organic technology on fungal microflora

Many of these microorganisms, such as *Pseudomonas fluorescens*, actinomycetes, *Trichoderma viride*, *Trichoderma hazianum*, *Paecilomyces marquandii* stimulated by the presence of ameliorative plants and organic mulch represented by wool and wood chips act as antagonists against soil borne pathogens of genus *Fusarium*, *Phytophthora* and *Alternaria*, producing a beneficial effect on the health of edible rose culture (Figures 3 and 4).

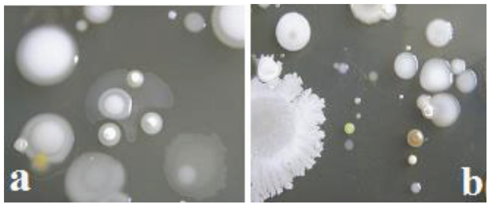


Figure 3. Bacterial microflora to V1.2. *Sinapis* with wool (a) and V7.1. *Tagetes* with wood chips (b)

Figure 4. Fungal microflora to V3.2. *Phacelia* with wool (a) and V7.1. *Tagetes* with wood chips (b)

Using ameliorative plants and different types of mulch in the organic technology for edible roses led to more dynamic global physiological activities of the soil microorganisms as reflected by a significant increase in soil respiration compared to controls, where recorded moderate values of released CO_2/100g dry soil (Figure 5).

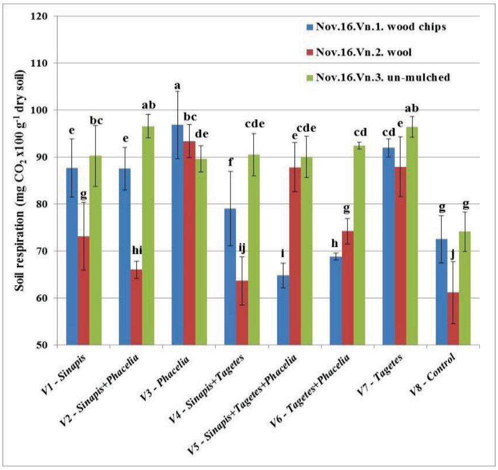


Figure 5. Influence of organic technology on soil respiration

The most intense metabolic activities were recorded at V3.1. *Phacelia* with wood chips sub-variant, mainly due to the activity of cellulolytic fungi, at V7.1. *Tagetes* with wood chips sub-variant mainly on account of bacterial activities (actinomycetes in particular) and cellulolytic fungi, followed by variants with *Tagetes* as ameliorative plant (based on fungal microflora) or combinations of two species of ameliorative plants.

In many of the variants with one or two species of ameliorative plants, soil respiration was more intense when it was used wood chips mulch compared to wool mulch but, in most cases, weaker or similar to the version un-mulched.

Figure 6 presents sectors of Pfeiffer chromate-grams for illustrating changes in soil quality under the influence of ameliorating plants (*Tagetes* and *Phacelia*) and mulch (wool and wood chips) compared to the control.

The analysis of chromatograms reveals an increasing silica organization due to biological activity though embattled shape of the outer edge of the central area in mulched variants, especially those with wood chips.

Clay shows most well-organized at the V3.1. *Phacelia* with wood chips sub-variant, the remaining variants presenting organization trends in different degrees of evolution, the organization level being correlate, in general, with the high level of chemical complexity.

The content of minerals increased significantly in V7.1. *Tagetes* with wood chips sub-variant, compared with the other experimental variants. Biological activity (bacterial and fungal), mineral diversity and enzyme activity is reflected in the organization of external and middle areas of the chromatograms, organizing corresponding to the increases of protein content, of the nutritional potential, of diversity of sources of carbon, of humic acids formation. Increases integration, connections between particles, due to increased dynamics of some processes compared with the control, but still insufficient to achieve a maximum level in the soil, possibly due to short action time of microbiota on the organic supplements. In the external area appear highlighted varied nutrient sources with an increase degree of stability, colloidal nature in the mulched variants and particularly better highlighted in the *Phacelia* variants. Formation of endings from the external area reflects the favorable evolution of organization of organic matter at variants with ameliorative plants and is more clearly evidenced at the variants mulched with wood chips.

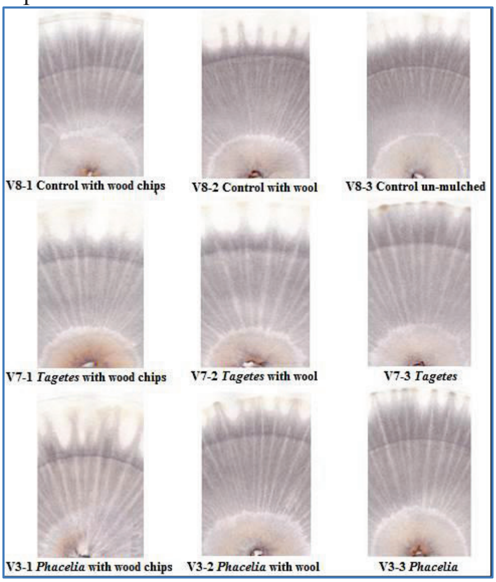


Figure 6. Soils chromatograms at the variants with ameliorative plants and mulch

CONCLUSIONS

Positive results through organic technology applied to edible rose cultures were obtained regarding stimulating the development of fungal and bacterial microflora and increasing the global physiological activities of edaphical microorganisms compared to controls and with the initial phases.

It is remarkable the beneficial effect of *Tagetes* alone or in combination with *Phacelia* on the development of bacterial and fungal microflora.

Using wood chips mulch determined a large numbers of bacteria developing on V7. *Tagetes* variant and fungi on V7. *Tagetes*, V3. *Phacelia* variants, were was recorded the most intense soil respiration also.

Using wool as mulch induced a weaker stimulation of soil microbial populations compared with wood chips, the best results being those related to soil respiration increase on V3. *Phacelia* or V7. *Tagetes* variants, or by the stimulation of bacterial increase in V1. *Sinapis* variant.

Analysis of chromatograms revealed favorable effect on soil quality evolution in mulched variants with ameliorative plants of *Tagetes* or *Phacelia*.

REFERENCES

Andersen M. K., 2005. Competition and complementarity in annual intercrops – the role of plant available nutrients. Department of Agricultural Sciences Environment, Resources and Technology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark, 3.

Berca M., 2011. Agrotehnică – Transformarea modernă a agriculturii. Ed. Ceres, București, 173.

Bergey D.H., Holt J.G., 1994. Bergey's manual of determinative bacteriology *9*, Wiliams and Wiliams Eds., Baltimore, USA, 787.

Butcaru A.C., Stănică F., Matei G. M., Matei S., 2016. Alternative methods to improve soil activity before planting an organic edible rose crop, Journal of Horticulture, Forestry and Biotechnology, Volume 20(4), 12-17, ISSN: 2066-1797.

Butcaru A.C., Stănică F., Matei G.M., Matei S., 2015. Pregătirea solului în vederea înființării unei culturi de trandafiri de dulceață în sistem ecologic, revista Hortus nr.14, 165-168.

Crossland M., Fradgley N., Creissen H., Howlett S., Baresel P., Finckh M. and Girling R, 2015. An online toolbox for cover crops and living mulches, Aspects of Applied Biology - Getting the Most out of Cover Crops, Volume 129, 1.

De Baets S., Poesen J., Meersmans J., Serlet L., 2011. Cover crops and their erosion-reducing effects during concentrated flow erosion. Catena Journal 85, ISSN 0341-8162 DOI: 10.1016/j.catena. 2011.01.009, 237–244.

Dhima K., Vasilakoglou I., Garane V., Ritzoulis C., Vaia Lianopoulou, Eleni Panou-Philotheou, 2010. Competitiveness and Essential Oil Phytotoxicity of Seven Annual Aromatic Plants. Weed Science 58,ISSN 1550-2759 DOI:10.1614/WS-D-10-00031.1, 457–465.

Domsch, K.H., Gams, W., 1970. Fungi in agricultural soils, T&A Constable Ltd., Edinburg, London, 290.

Hooksa C.R.R., Wangb K., Ploegc A., Mcsorleyd R, 2010. Using marigold (*Tagetes* spp.) as a cover crop to protect crops from plant-parasitic nematodes. Applied Soil Ecology 46, ISSN 0929-1393, 307–320.

IFOAM EU GROUP, 2010. Organic food and farming. A system approach to meet the sustainability challenge. www.ifoam-eu.org/workareas/policy/php/CAP.php, 7-8.

Liu J., Khalaf R., Ulén B., Bergkvist G., 2013. Potential phosphorus release from catch crop shoots and roots after freezing-thawing. Plant Soil Journal, electronic ISSN 1573-5036 DOI 10.1007/s11104-013-1716-y.

van Opheusden A.H.M., van der Burgt G.J.H.M., Rietberg P.I., 2012. Decomposition rate of organic fertilizers: effect on yield, nitrogen availability and nitrogen stock in the soil, Louis Bolk Institute, www.louisbolk.org, 33.

Penhallegon R., 2003. Nitrogen-phosphorus-potassium values of organic fertilizers. OSU Extension Service - Lane County Office, Karen Ailor, http://extension.oregonstate.edu/lane/sites/default/files/documents/lc437organicfertilizersvaluesrev.pdf, 4.

Reeve J. R., 2007. Soil quality, microbial community structure, and organic nitrogen uptake in organic and conventional farming systems. Washington State university; Department of Crop and Soil Sciences;http://www.researchgate.net/publication/228542927.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOME ASPECTS REGARDING FLOWER'S MORPHOLOGY ON SEVERAL LOCAL POPULATION OF *PRUNUS DOMESTICA* L. FROM PATÂRLAGELE (BUZĂU COUNTY)

Daniel Constantin POTOR, Mihaela Ioana GEORGESCU, Dorel HOZA

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: danutpotor@yahoo.com

Abstract

According to the literature, a number of morphometric values of flower from varieties of Prunus domestica may be correlated with their degree of fertility. We chose four local populations of plums from Pătârlagele area, Buzau County, to measure the floral components - sepals (length), petals (length), stamens (number and length), carpel (length) and to compare them with the literature. The results of our observations shows the presence of a local variability regarding the perianth, the average number of stamens, the average length of the carpel and the ratio between the number of stamens and the length of the carpel; for the T3 and T4 populations there are slight differences from literature on the average length of petals. The ratio of average number of stamens and carpel length shows low values for the four populations (range 1.35 to 1.63) compared with the data from literature, indicating the possible presence of self-incompatibility among the four local populations of Prunus.

Key words: *Prunus domestica* L., *flower components, self-incompatibility, local populations.*

INTRODUCTION

Research on the variability of infraspecific characters of the *Prunus domestica* was aimed primarily towards fruit morphology (Buia, 1956), biology of flowering (Branişte, 1994), morphology, viability and pollen germination (Gilani et al., 2010; Calicut et al., 2013) or degree of fertility of varieties (Ionita, 1956; Boredianu et al., 1965; Cociu, Bumbac, 1985). The differences in the morphology of the flower are fairly low compared to the general characterization of higher taxa; Flora RPR, 1956 shows the following characters for *P. domestica* flower: sepals' length 2.5-5 mm; petals' length 7-12 mm.

In a comparative analysis of variation of the morphological characters of the flower to 23 plum varieties, Mădălina Butac (2003) shows that they have been grouped into 12 classes according to the diameter of the flower, in 9 groups depending on the average length of the pistil, respectively in 9 groups after ratio between the number stamens and pistil length. Analysing these characters in relation to the fertility of the varieties it has been observed that the self-fertile and partly self-fertile varieties have the flower's diameter, the length of the pistil, the number of stamens and the ratio of the number of stamens and length pistil have much higher values and significantly different from those with self-incompatibility.

MATERIALS AND METHODS

Pătârlagele city is located in the north - west of Buzau County, at 45 ° 19 ' north latitude and 26 ° 21' east longitude, at a distance of 56 km from the city of Buzau. The city is located in the Pătâragele Basin from the Curvature Subcarpathians, on the Buzau River, at an altitude of about 400 meters, dominant landscape of the area consisting of hills.

It were selected 4 local populations, denoted T1, T2, T3 and T4, located in plantations set in the village. Populations of T1, T3, and T4 are in independent plantations, while T2 population is planted alongside other local varieties. The land with plantations made by T2, T3, T4 populations has north exposure while the T1 population is on a land with south exposure.

For the morphological characterization of the flower (Figure 1) the following indicators were chosen: the average length of the sepals, the average length of the petals and the ratio between them, the average number and length of the stamens, the average length of the carpel, the ratio between the length of the carpel and of

the petals and the ratio between the number of stamens and length of carpel. From each population were taken twenty five flowers into flowering phenophase.
Results were used for comparative analysis of the four populations.

Figure 1. Flower of *Prunus domestica* population T3

RESULTS AND DISCUSSIONS

The morphometric measurements on T1 flowers variety (Table 1) shows that the petals have an average length of 9.16 mm and are about 2.14 times longer than sepals; average number of stamens is 19 and the carpel's average length is 12.90 mm; it exceeds the flower perianth of 1.41 times; the ratio between the average number of stamens and carpel average length is equal to 1.41.

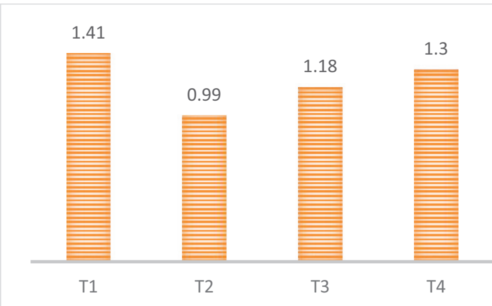


Figure 2. The ratio between the average length of the carpel and of the petals

The values of the floral components of the T2 population (Table 1) are close to the followings floral indicators of T1 population: sepals' average length (4.13 mm), average number of stamens (19.5) and average carpel length (12 mm); the size of the petals is obviously higher (12.16 mm) while the ratio between them and the average size of the carpel is lower (0.99).

The ratio between the average number of stamens and the average length of the carpel is 1.63 (Figure 3) is the highest value recorded for the 4 populations.
In the group of the four populations, the flowers from the T3 population (Table 1) shows the highest values to the average length of sepals (4.79 mm), petals (12.80 mm), carpels (15.10 mm) and average number of stamens (22); the value of the ratio between the average length of petals and carpel (Figure 2) is the smallest of the observed populations series; also, the ratio between the number of stamens and the average length of carpels - 1.46, is at the middle of the series of values for the 4 populations (Figure 3).

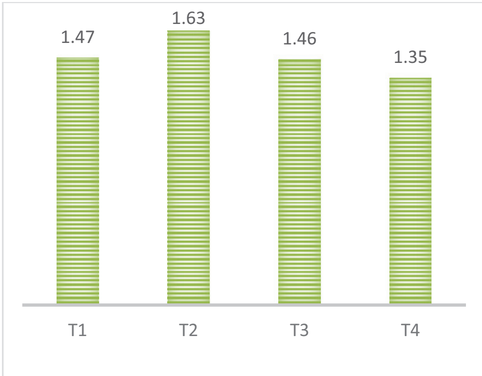


Figure 3. The ratio between the average number of stamens and the average length of carpel

The lowest values for the used indicators are found to the morphometric measurements of floral components of T4 population (Table 1), except the average length of the carpel (12.20 mm) and the ratio between the average length of carpel and the petals (Figure 2).
Comparing the results obtained with literature data it is observed that:
- The length floral perianth components fall within the values indicated in the description of the *Prunus domestica* flower (Romanian Flora, 1956), except populations T2 and T3 which the average length of petals slightly exceeds data from literature.
- For the T3 population there are values of floral components that exceeding the data in the literature like the average length of the carpel, while the remaining variants fall between the average values of the literature (Butac M., 2003).

- The ratio of average number of stamens and carpel length set shows low values for the four populations (range 1.35 to 1.63) compared with the data from literature (range 1.42 to 2.33) (Butac M., 2003).

Table 1. Morphometric characters of the floral components of *Prunus domestica* populations from the area Pătârlagele (Buzau County)

Population	Sepals - average length (mm)	Petals - average length (mm)	Petals/sepals - average length ratio	Stamens - average number	Stamens - average length (mm)	Carpel - average length (mm)	Carpel/petals - average length ratio	Stamens average number / carpel average length
T1 (Prun roșu)	4.28	9.16	2.14	19	7.99	12.90	1.41	1.47
T2 (Gras)	4.13	12.16	2.94	19.5	9.95	12	0.99	1.63
T3 (Gras CB)	4.79	12.80	2.67	22	8.96	15.10	1.18	1.46
T4 (Ciorăsc)	3.63	9.38	2.58	16.50	6.59	12.20	1.30	1.35

CONCLUSIONS

The four local populations of *Prunus domestica* examined in terms of the morphology of the floral components shows some differences with the data in the literature, and that indicated the existence of local variability on the flower's level.

The size of the flower's perianth and the ratio between the average number of stamens and length of carpel indicate that in the analysed populations, self-incompatibility and self-sterility may be present.

REFERENCES

Branişte N., 1994. Polenizarea şi fecundarea la prun. Mapă documentară, nr 39.

Buia Al., 1956. Familia Rosaceae. In Flora RPR vol. IV, Ed. Acad. RPR, Bucureşti, 835-834.

Butac Mădălina Maria, 2003. Biologia înfloritului şi fructificării soiurilor de prun din sortimentul naţional în scopul stabilirii celor mai buni polenizatori. Teză de doctorat. Bucureşti, 145.

Ćalić, D., Devrnja, N., Kostić, I., & Kostić, M., 2013. Pollen morphology, viability, and germination of *Prunus domestica* cv. Požegača. Scientia Horticulturae, 155, 118-122

Gilani, S. A., Qureshi, R. A., Khan, A. M., & Potter, D., 2010. Morphological characterization of the pollens of the selected species of genus *Prunus* Linn. from Northern Pakistan. African Journal of Biotechnology, 9 (20), 2872.

Ioniţă C., 1956, Contribuţii la studiul fertilităţii soiului de prun Tuleu Gras.Grădina, via şi livada, nr. 5.

Săvulescu T., Beldie AL., Buia AL., Guşuleag M., Nyarady E., Prodan I., Răvăruţ M., 1956. Flora Republicii Populare Române IV, Editura Academiei Republicii Populare Române.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CANOPY MANAGEMENT PRACTICES IN MODERN PLUM (*PRUNUS DOMESTICA* L.) PRODUCTION ON VIGOROUS ROOTSTOCKS

Miljan CVETKOVIĆ[1], Gordana ĐURIĆ[1,2], Nikola MICIC[1,2]

[1]University of Banja Luka, Faculty of Agriculture, Bulevar Vojvode Petra Bojovića 1A, 78000 Banja Luka, Bosnia and Herzegovina
[2]University of Banja Luka, Genetic Resources Institute, Bulevar Vojvode Petra Bojovića 1A, 78000 Banja Luka, Bosnia and Herzegovina

Corresponding author email: miljan.cvetkovic@agrofabl.org

Abstract

Intensive high-density plantings (HDP) of plum trees in the Republika Srpska involve the use of Myrobalan (Prunus cerasifera Ehrh.) seedling as the predominant and, in most cases, the only rootstock. Using Myrobalan as a vigorous rootstock is a serious challenge in growing plums at higher planting densities. Although Myrobalan seedling rootstock increases the vigour of grafted cultivars, plum trees trained to the spindle system on Myrobalan rootstock can also be grown at very high plant densities ranging from 1,000 to 1,800 trees per hectare, depending on the cultivar/rootstock combination and central-leader inclination. The most common training system for plums in high density plantings is the slender spindle or the spindle bush system. Successful training and maintenance of spindle systems in intensive production on high-vigour rootstocks is not possible without the consistent use of canopy management practices, particularly during the first three years after planting, when these practices are most intensive for proper training of both the central leader and main lateral branches. Canopy management practices require a professional attitude and substantial manual labour. Particular importance in training spindle systems for plums as well as in maintaining the training system (replacement of spur-bearing branches) is given to the following specific canopy management practices: notching, shoot bending, shoot twisting, undercutting and replacement of spur-bearing branches. This paper outlines some important canopy management practices and their effect on plum growth and development, focusing on cultivar-specific responses to treatments.

Key words*: plum, canopy management practices, cultivar.*

INTRODUCTION

On an annual level, plum production in Bosnia and Herzegovina (during 2003 – 2013) showed an increase in total land area (+7.37%) and production (+4.80%) and a decline in average yield (–2.39%) (FAOSTAT, 2016). Although plum is the leading fruit crop in BiH (Statistics Agency, BiH), the intensity of production is rather low, which may be associated with the use of growing methods (Mićić et al., 2005) unadapted to the tendency to introduce new cultivars into production.

The most common training system for plum trees in high density plantings (HDPs) is the slender spindle or spindle bush (Grzyb and Rozpara, 1998; Hrotko et al., 1998; Meland, 2001; Čmelik et al., 2002; Gavrilescu et al., 2004) which uses low-vigour rootstocks. Establishing new highly intensive (high density) plum plantings in Bosnia and Herzegovina characteristically involves the use of Myrobolan (*Prunus cerasifera* Ehrh.) seedling as the predominant, and in most cases the only rootstock available for plum grafting. In modern highly intensive plantings under high density planting systems which use higher vigour rootstocks, practices designed for canopy management during dormancy and timely summer pruning operations are the preconditions for successful plum production (Mićić et al., 2005; Milošević et al., 2008; Glišić, 2012; Cvetković et al., 2015).

Shoot management operations aimed at creating the best crotch angle possible such as shoot bending (during the first part of the growing season) can have an important effect on generative bud differentiation and, hence, facilitate the control of growth and development processes.

The objective of this study was to analyze shoot bending in plum trees grown on vigorous rootstocks in highly intensive plum production systems.

MATERIALS AND METHODS

The analysis of shoot bending practices in plum trees was conducted in a plum planting at Gunjevci (44°35'33"; 18°56'38") near Kozarska Dubica (BiH) at an altitude of 155 m.
The planting was established in autumn 2009. The experimental plot has a north-western exposure, with a slight inclination (2%). Total land area of the planting is 1 ha.
Four cultivars were planted: 'Stanley', 'Čačanska Lepotica', 'Čačanska Najbolja' and 'Čačanska Rana'. All cultivars were grafted on Myrobalan (*P. cerasifera* Ehrh.) seedling rootstock.
Trees were trained to the spindle system using all necessary canopy management practices. Spacing for all cultivars was 1.5 m within the row and 4.0 m between rows.
The planting received standard cultural practices. Soil management systems were grass mulch for the inter-row space, and bare fallow combined with herbicide band for the within-row space.
The research was carried out in 2010 - 2013. The trees of the tested cultivars were subjected to shoot bending, as follows:
a) initial spreading of the shoots to keep them at an angle subordinate to the central leader – at the beginning of the growing season, and
b) bending of the shoots to retain the desired position relative to the central leader – in the middle of the growing season.
Shoot management treatments were applied in the entire planting, but 30 trees per cultivar subjected to shoot bending operations were randomly selected for detailed analysis.
The shoots were initially spread out by wooden toothpicks, whereas in the second part of the growing season they were bent by plastic hooks, twine (aluminium wire and plastic wire spreaders) and twisting.
After the three–year experimental period, the shoot bending techniques used in the study were analysed for visual shown and integrated evaluation of their efficiency and effectiveness.

RESULTS AND DISCUSSIONS

Spreading of the shoots. The initial spreading of the shoots along the central leader by toothpicks in the tested cultivars (Figures 1a and 1b) favoured the formation of a proper crotch angle (about 90°) of the newly formed shoots which generally provide the basis for the spindle structure in plum trees (Lučić et al. 1996; Mićić et al., 2005; Gonda, 2006; Glišić, 2012).

Figure 1. Spreading of shoots by toothpicks - variety ’Čačanska Lepotica’ (a) and 'Čačanska Rana' (b)

The spreading operation in the tested cultivars led to reduced apical dominance (Wilson, 2000). Importantly, spreading by toothpicks should be done successively in accordance with the shoot growth dynamics.
The shoots subjected to spreading exhibited a higher rate of generative bud differentiation (Mićić et al., 1998). The horizontal position of the shoot resulted in higher percent activation of growth points along the shoot during the same or following growing season, with shorter growth formed and much of this growth

developing into fruiting wood. Such a response was effective in reducing growth vigour and producing a more favourable ratio of vegetative to generative growth on the tree, which is of particular importance in the first years of plum production on higher vigour rootstocks.

The spreading efficiency of toothpicks is dependent on the optimum time to use them. Spreading should be performed when the shoot has reached a length of 15 – 20 cm, which is, in part, a cultivar-specific trait.

In 'Čačanska Najbolja' and 'Čačanska Rana', spreading should be applied to shorter lengths of shoots. 'Čačanska Najbolja' exhibits intensive growth, and its shoots longer than 20 cm generally have a greater base diameter and are not spreadable. 'Čačanska Rana' showed a tendency to develop shorter shoots with a higher rate of lignification at the base, thus potentially creating spreading problems. 'Stanley' and, particularly, 'Čačanska Lepotica' show a positive response to shoot spreading by toothpicks even at a later developmental stage. Mitrović et al. (2005) and Glišić et al. (2007) agree that shoot bending is a mandatory practice in establishing dense plum plantings on vigorous rootstocks, but no precise shoot bending times are defined in their studies. Toothpicks should be used in succession – only shoots that have reached the required developmental stage are to be spread out.

Previous experience has shown a very positive effect of toothpicks (Mićić et al., 2005; Glišić et al. 2007; Glišić, 2012*)*. If the shoots are bent in the second part of the growing season without being previously spread at this early stage, the so–called "knee" is formed at the shoot base.

The use of toothpicks has a number of advantages: a high installation efficiency rate (a large number of toothpicks installed per unit time); good spreading performance; simple installation; causing minimum damage to the tissue which shows a high healing rate; the natural material they are made of has no negative effect on plants; they are available on the market and very affordable.

Problems with toothpick use generally include their post-installation instability, which may be due to low toothpick quality, improper installation or adverse weather conditions (heavy rain, wind) after installation. If improperly installed, toothpicks soon fall off, and the treatment must be repeated for satisfactory spreading performance.

Spreading requires intensive manual labour continuously throughout the growing season (until mid-July), which may be a constraint to the use of this practice in large plantations. Upon use of toothpicks to spread out shoots during the initial stage of shoot development and crotch angle formation in the tested cultivars, most of the shoots exhibiting high growth vigour continue their intensive growth in the middle of the growing season. In order to hold the shoots in position, they were further spread by plastic hooks (hereinafter referred to as hooks), aluminium wire and plastic twine shortly before shoot lignification at the base (Figure 2 a,b,c).

The use of hooks to spread shoots during the growing season showed a range of practical advantages: relatively easy installation; hooks can also be used to spread shoots that show strong growth; relatively easy removal after use; hooks can remain on the tree for use in the following growing season (which might cause their partial deformation); they cause no damage to either the leader or the shoot.

Although they increase production costs to some extent, these types of hooks have also been manufactured in the domestic market in the last years at a relatively affordable price.

When purchasing hooks, it is advisable to pay attention to their resistance to UV radiation.

The use of aluminium wire (and plastic twine) for shoot bending in this research showed the following disadvantages: relatively low spreading efficiency per unit time (the wire cannot be cut to a required length far in advance of the spreading operation, but rather shortly before the treatment; this slows down the operation and the process of finding a suitable position on both the shoot and the leader or some other type of growth to which the wire is to be fastened – this was a problem especially with plastic twine which was fastened to two positions; a crotch angle of 90^{o} is difficult to establish by wiring and twining in shoots being spread at the base (unless previously done by toothpicks); wires can often fall off after installation, especially at the point of contact with the shoot, and the operation requires correction (which was not the case with the plastic twine); during the growing season, wires must be removed from vigorous shoots to prevent them from cutting into the shoot tissue (in more extreme situations, this can lead to breakage of the shoot and, later on, the year–old branch), which is also a problem with plastic twine.

Wires are given priority in spreading strong shoots that are unspreadable by hooks, if no twisting is used.

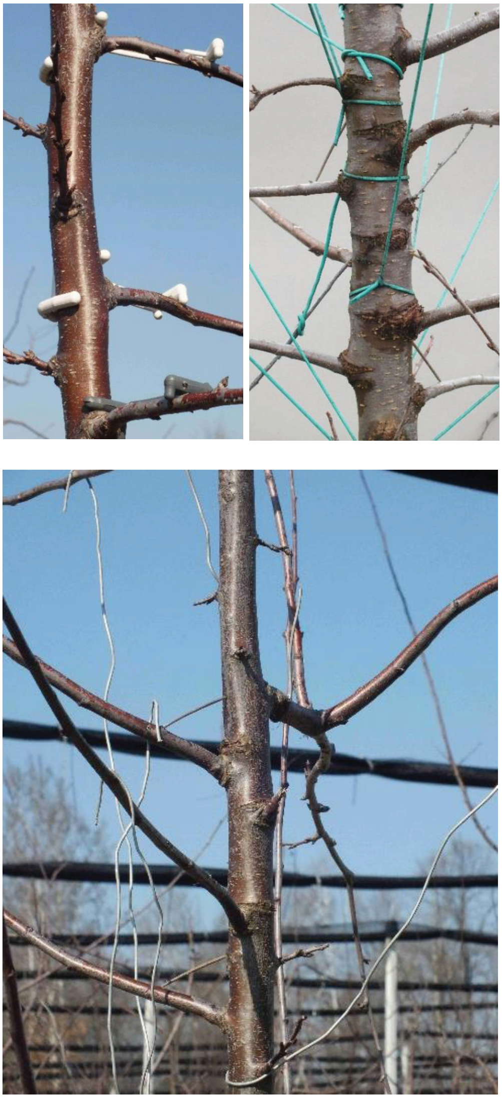


Figure 2. Spreading of shoots by plastic hook (a -variety ’Čačanska Lepotica’), plastic twine (b - variety ’Stanley’) and aluminium wire (c - variety ’Čačanska Rodna’)

Shoots that have been timely brought to a proper position in relation to the central leader will by the end of the growing season acquire the character of the fruiting wood i.e. mixed–type fruiting branch or, rarely, long vegetative growth.

Shoot twisting. The vigour of a plum tree, especially when Myrobolan is used as a rootstock, very often induces intensive shoot growth in the second part of the growing season, showing the tendency for apical growth, even if hooks or wire spreaders are previously used. In order to keep the shoots subordinate to the central leader and prevent apical dominance, shoot twisting was performed on the tested cultivars in early July.

The operation was aimed at reducing the vegetative growth activity of the shoots and inducing flower bud differentiation. Shoot twisting was done in accordance with the principles set down by Mićić et al. (2005), which ensure satisfactory operation and adequate performance: at twisting, the shoots had active tip growth; twisting involved holding the shoot base in one hand, while twisting the shoot over a wide area with the other hand, to prevent breakage; twisting was repeated if it failed to produce the desired effect in the first twisting treatment. In shoots exhibiting lower growth vigour and smaller base diameter, twisting entails smaller spacing between the hands i.e. the portion of the shoot subjected to the twisting pressure is shorter.

When twisting stronger shoots with greater base diameter, the spacing between the hands is wider, and the portion of the shoot subject to the twisting pressure is longer. This is common in shoots subjected to twisting later than considered optimum (degree of lignification of the shoot base) or which are not spread out in initial developmental stages (by either toothpicks or hooks). The twisting pressure was applied until creases appeared on the bark, and in certain cases until the bark was damaged or cracked. So far, research on the use of twisting has shown that bark damage due to twisting does not have an adverse effect on further growth and development (resin flow, shoot dieback) although this may be an open question from the viewpoint of the ecological and health situation of the trees (Figure 3 a,b,c).

The present research has confirmed that the damaged portion of the shoot shows very good healing in biological terms, and does not differ from the rest of the shoot. The position of the shoot when released speaks of the twisting performance.

The return of the shoot to its original position is a reliable sign of improper twisting. Strong twisting pressure which brings down the shoot completely results in complete crushing of growth vigour and fruit bud formation along the shoot.

Figure 3. Results of shoot twisting (a - variety ’Čačanska Lepotica’, b - variety ’Stanley’) and aluminium wire (c - variety ’Čačanska Rodna’).

This is in favour of the conclusion that greater efficiency is always achieved by strong twisting. Twisting plays an important role in controlling vegetative growth and initiating cropping in initial years of spindle formation. Glišić (2012) observed that the combined use of canopy management practices, with twisting included, leads to trees coming into bearing very early and reaching full productivity at an earlier date in a considerable number of plum cultivars grown under high density system.

Twisting in other stone fruit crops is not so efficient (sour cherry) as in plums or it leads to excessive shoot damage or dieback (sweet cherry).

CONCLUSIONS

Plum production on Myrobalan (*Prunus cerasifera* Ehr.) seedling rootstock under spindle training and high density planting systems requires proper and timely tree management practices during the growing season, along with the need to follow the rest of intensive production principles.

Shoot management operations during summer pruning are designed to bring the shoots at their initial developmental stages to the best position possible in relation to the central leader and enable crushing of apical dominance and vegetative vigour.

Initial spreading by toothpicks at the beginning of the growing season and subsequent bending in the second part of the season provide optimum spreading performance.

Under intensive growth conditions in the second part of the growing season, high efficiency is achieved by shoot bending as well. Cultivar-specific response to these treatments can influence the date, effectiveness and efficiency of the treatments.

REFERENCES

Cvetković M., Mićić N., Đurić G., Bosančić B., 2015. Leader management techniques to induce vegetative bud development in plum. In: A. Papachatzis and Damiano Avanzato (eds) Book of abstracts. 3rd Edition. Present constains of Plum Growing in Europe. Skopelos: 28.

Čmelik Z., Duralija B., Bencic D., Druzic J., 2002. Influence of rootstocks and planting density on performances of plum trees. Acta Horticulturae, 577: 307-310.

Gavrilescu E., Cosmulescu S., Baciu A., Botu M., 2004. The influence of cultivar-rootstock combinaton on dinamical physiological proces in prune species. In: 8th International Symposium on Plum and Prune Genetics, Breeding and Pomology. Book of Abstracts, 8(112E).

Glišić I., Milošević T., Glišić S.I., Milošević N., 2007. Određivanje optimalnog termina za izvođenje zahvata rovašenja kod nekih sorti šljive. Zbornik naučnih radova 22. Savetovanja Unapređenje proizvodnje voća i grožđa, Grocka, 13(5): 41-46.

Glišić I., 2012. Pomotehničke specifičnosti šljive gajene u gustoj sadnji. Thesis. Faculty of Agronomy, Čačak.

Gonda I. 2006. The role of pruning in the intensification of plum production. International Journal of Horticultural Science, 12(3): 83-86

Grzyb Z.S., Rozpara E., 1998. Plum production in Poland. Acta Horticulturae, 478: 19-24.

Hrotkó K., Magyar L., Simon G., Klenyán T., 1998. Effect of rootstocks on growth of plum cultivars in a young orchard. Acta Horticulturae, 478: 95-98

Lučić P., Đurić G., Mićić N., 1996. Voćarstvo I. Institut za istraživanja u poljoprivredi Srbija: Nolit-Partenon.

Meland M., 2001. Early performance of european plum high density production systems. Acta Horticulturae, 557: 265-274.

Mićić N., Đurić G., Cvetković M. 2005. Sistemi gajenja i rezidba šljive. Ministarstvo poljoprivrede, šumarstva i vodoprivrede Republike Srbije, Beograd, 1-60.

Mika A., 1992. Trends in fruit tree training and pruning systems in Europe. Acta Horticulturae, 322: 29-36

Milosevic T, Zornic B, Glisic I., 2008. A comparison of low-density plum plantings for differences in establishment and management costs, and in returns over the first three growing seasons - a mini-review. Journal of Horticultural Science and Biotechnology, 83:539-542

Mitrović M., Blagojević M., Karaklajić-Stajić Ž., Rakićević M., 2005. Sistem guste sadnje u savremenoj tehnologiji gajenja šljive. Traktori i pogonske mašine, 10(2): 103-107.

Wilson BF., 2000. Apical control of branch growth and angle in woody plants. American Journal of Botany, 87: 601-607

http://faostat3.fao.org/browse/Q/QC/E (site visited 20.06.2016)

http://www.bhas.ba/saopstenja/2016/AGR_2015_001_01-BS.pdf (site visited 20.06.2016)

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

GROWTH AND FRUITING POTENTIAL OF SOME APPLE VARIETIES WITH GENETIC RESISTANCE TO DISEASE, GROWN IN HIGH DENSITY SYSTEM

Gheorghe PETRE[1], Daniel Nicolae COMĂNESCU[1], Adrian ASĂNICĂ[2]

[1]Research and Development Station for Fruit Growing Voinesti, 387 Main Street, 137525, Dambovita, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: statiuneavoinesti@gmail.com

Abstract

The research conducted at the Research and Development Station for Fruit Growing Voinesti in the period 2014-2016 highlights the growth potential of apple varieties with genetic resistance to diseases such as: 'Ariwa', 'Golden Lasa', 'Goldrush', 'Enterprise', 'Inedit', 'Iris', 'Luca', 'Real', 'Rebra', 'Redix', 'Remar', 'Saturn', 'Voinicel', all grafted on the M9 dwarfing rootstock. Orchard is defined as high density system with 2,500 trees/ha. The growth vigor of the ten years old trees, when the growth potential is well defined, indicates that between apple varieties with genetic resistance to the diseases under study, were significant differences noticed in the thickness of the trunk, size of the trees and crown volume. The production potential of apple varieties ranged from 28 to 44 t/ha in trees aged of 8-10 years. Most varieties yield over 30 t/ha, considered as apple varieties with high production potential. The level of costs for phytosanitary treatments in apple varieties with genetic resistance to disease is lower with more than 55% compared to the sensitive varieties like 'Jonathan', 'Golden Delicious', 'Starkrimson'.

Key words*: Vf resistance, productivity, economic efficiency, environment.*

INTRODUCTION

Apple's assortment has seen a significant change over the last decades, with varieties dedicated to meet growing consumer demands (Petre et al., 2005). A special situation is the promotion of apple varieties with genetic resistance to diseases, which for the new plantations are the key to the efficient economic technology with immediate effect by the total or partial elimination of the fungicide treatments (Comanescu et al., 2012).

The gradual change of the assortment through the promotion of varieties of apple with genetic resistance to diseases depends on certain characteristics that they can exhibit (Comanescu, 2002). The superiority of new varieties should mainly address the requirements of the producer, oriented to economic efficiency, vigour of growth, high production potential, fruit appearance, consumption period, as well as the taste of the consumer (Petre et al., 2014, 2015).

The important advances made in the research of apple varieties with genetic resistance to diseases at RDSFG Voineşti allowed study of 13 scab resistant varieties behaviour in the orchard, with 'Jonathan', as sensitive to diseases control variety.

MATERIALS AND METHODS

The research was organized in an apple orchard belonging to RDSFG Voineşti, Experimental Base no. 1, between 2014 and 2016, and highlights the potential for growing and fruiting of the apple varieties with genetic resistance to diseases, representing the main factor in obtaining of an adapted production to the requirements of European quality standards. We have studied 13 varieties with genetic resistance to diseases, namely 'Ariwa', 'Golden Lasa', 'Goldrush', 'Enterprise', 'Inedit', 'Iris', 'Luca', 'Real', 'Rebra', 'Redix', 'Remar', 'Saturn', 'Voinicel' compared to sensitive apple variety 'Jonathan'. All apple varieties were grafted on M 9 rootstocks. The trees were planted in 2007 at a distance of 4 x 1m (2,500 trees / ha), trained as slender spindle (Figure 1).

Figure 1. Experimental field with scab resistant varieties trained as slender spindle

Observations and determinations have been made regarding the growth vigour expressed in in trunk thickness and tree crown size, production record, and average fruit weight.

The orchard's soil is brown eumesobasic, pseudogley, with a pH ranged between 5.7 - 5.9. Humus content is medium in the upper part (2.0 - 2.9%), medium supplied with nitrogen and poorly supplied in phosphorus and potassium.

The climatic conditions were favourable for growing and fruiting of trees, characterized by an average annual temperature higher than 1.9^0C, with an annual precipitation amount of 755 mm.

In the orchard, natural grass was maintained mowed between the rows and clean along the tree rows. To control pests 6 to 8 treatments were applied only with insecticides.

RESULTS AND DISCUSSIONS

Trees Growth

The vegetative growth of trees is determined by a number of biological factors such as variety, rootstock, disease and pest resistance, but also technological: fruit load, optimal provision of technological measures, as well as nutrition and water conditions necessary for the development of the physiological processes.

The vigour of apple varieties trees with genetic resistance to diseases is manifested in quantitative terms by the annually volume of vegetative growth, trunk size, height and size of the tree crown. These parameters are depending on the vigour of the varieties, corroborated with the degree of fertility of the soil, planting distance etc.

According to the diameter of the tree trunk, 10 years after planting, the scab resistance apple varieties grown in a high density system and grafted on the M9 rootstock rank as follows (Table 1):

- vigorous varieties with trunk diameters above 80 mm: 'Luca' (96.18 mm), 'Golden Lasa' (80.40 mm), 'Enterprise' (87.90 mm), 'Rebra' (81.62 mm), 'Redix' (87.23 mm), 'Remar' (85.90 mm);
- medium vigorous varieties, with trunk diameters between 65-80 mm: 'Iris' (67.35 mm), 'Inedit' (70.50 mm), 'Voinicel' (67.60 mm), 'Real' (67.50 mm), 'Ariwa' (68.03 mm);
- low vigour varieties with trunk diameters below 65 mm: 'Saturn' (62.39 mm), 'Goldrush' (55.44 mm).

Table 1. Vegetative growth of apple varieties trees cultivated in high density system (2,500 trees / ha)

No	Variety / M9	Diameter (mm)		Tree size (cm)		Crown volume		Dif. ± control
		value	Average growth increase	Height	Thickness of the fruiting fence	mc/tree	mc/ha	
1	Ionathan (control)	66.38	5.79	245	130	2.53	6,325	-
2	Golden Lasa	80.40	7.17	240	140	2.66	6,650	+325
3	Ariwa	68,03	6.35	250	135	2.70	6,750	+425
4	Goldrush	55.44	4.74	230	120	2.16	5,400	-925
5	Enterprise	87.90	6.57	270	150	3.30	8,250	+1,925
6	Inedit	70.50	6.20	230	120	2.16	5,400	-925
7	Iris	67.35	6.50	205	135	2.09	5,225	-1,100
8	Luca	96.18	8.59	270	145	3.19	7,975	+1,650
9	Real	67.50	6.60	250	130	2.60	6,500	+175
10	Rebra	81.62	7.43	275	140	3.15	7,875	+1,550
11	Redix	87.23	7.53	270	140	3.08	7,700	+1,375
12	Remar	85.90	7.80	260	135	2.83	7,075	+750
13	Saturn	62.39	4.43	230	130	2.34	5,850	-475
14	Voinicel	67.60	5.30	230	130	2.34	5,850	-475

The average growth of the trunk, in the 10th year since planting, exceeded 7 mm in the varieties: ‘Golden Lasa’, ‘Luca’, ‘Rebra’, ‘Redix’ and ‘Remar’, and below 7 mm varieties such as ‘Inedit’, ‘Iris’, ‘Ariwa’, ‘Enterprise’, ‘Saturn’, ‘Real’, ‘Voinicel’ and ‘Goldrush’, which recorded an increase in growth between 4.43 - 6.60 mm.

The tree crown volume provides the skeleton to support the branches, leaves and fruits. Depending on the type of orchard, crown volume differs, both at the tree level and at the surface unit level. Through its structure, the crown's volume must ensure that the light penetrates all the tree elements, a prerequisite for maintaining its garnish with fruit branches to produce as much productive volume as possible.

The volume of the crown depends on the size of the trees, which is influenced to a large extent by the vigour of the variety. Based on the vigour of the trees, the crown volume was calculated at the tree level and at surface unit.

Regarding the crown volume recorded in the 10th year of planting at the tree level, it oscillated with quite large variation between varieties, from 2.09 cm / tree to ‘Iris’, ‘Goldrush’ and ‘Inedit’ variety 2.16 cm / tree, up to 3.30 cm / tree in the ‘Enterprise’ variety. Larger volumes were remarked at ‘Enterprise’, ‘Luca’, ‘Ariwa’, ‘Real’, ‘Golden Lasa’, ‘Rebra’, ‘Remar’, ‘Redix’, with the crown volume on the tree of more than 2.5 cubic meters.

The volume of the crown calculated on the surface unit follows the same grading of varietal vigour, given by the planting density of 2500 trees/ha for all varieties. Thus, the largest crown was recorded in the ‘Enterprise’ variety - 8,250 mc / ha and the lowest in ‘Iris’ varieties with 5,225 and ‘Goldrush’ with Inedit 5,400 mc / ha respectively.

Lower values of the crown volume at the surface unit were also recorded for the apple varieties ‘Saturn’ and ‘Voinicel’ with values of 5,850 mc / ha.

In terms of vegetative growth, small and medium-sized varieties are suitable for high-density orchards at 4 x 1 m planting distances with 2,500 trees / ha.

Trees Productivity

One of the main goal of the research is the evaluation of apples production capacity, a very strong reason for new modern orchards.

The high productive potential associated with the superior quality of fruit expresses the highest degree of genetically-resembled ability of scab apple varieties in the ecological conditions of the area in which they are grown.

The productivity of apple varieties with genetic disease resistance, which is the subject of this study, is a complex, genetically determined hereditary base from which it originates, but is influenced by the interaction between the variety and the climatic conditions of the area of culture. Other factors contributing to the mapping of this attribute of genetically resistant apple varieties are related to the precocity of the fruit, the type of fructification, the applied technology, the resistance to diseases and pests, the compatibility to grafting and pollination, the density of planting and the rootstock used.

For the correct analysis of the harvest quantity, it was envisaged to record the fruit production each year and to determine the level of production, the self-regulation or the intermittent fructification trend.

The annual record of apple production at the variety level shows that there are differences in production levels.

For the appreciation of the productivity of the varieties, the production recorded in the 8-10 years from planting was taken into account (Table 2).

The yield obtained in the 8th - 10th years from planting show the outstanding performance of the apple-high-density system, which can be expanded into well-established fruit-growing areas, only with the most productive varieties, which give quality fruit adapted to market demands.

In the 8th leaf, the largest productions were recorded for the varieties ‘Real’, ‘Remar’, ‘Iris’, ‘Luca’, ranging from 38.8 to 46.8 t/ha. Most varieties recorded over 30 t/ha, including Jonathan.

In the 9th year after planting, the highest yields were obtained from ‘Ariwa’, ‘Iris’, ‘Real’, ‘Remar’ and ‘Saturn’, ranging from 34 to 38 t/ha. The other varieties recorded yields ranging from 24.7 to 29.5 t/ha, compared to the Jonathan, which was recorded 22.5 t / ha.

Table 2: Production of apple varieties with genetic resistance to diseases, cultivated in a high density system (2,500 trees / ha), in the 8-10 years leaf

No	Variety	Yield (t/ha)				Dif. ± Control
		8th year / 2014	9th year / 2015	10th year / 2016	Average	
1	Ionathan (control)	30.3	22.5	24.3	25.7	-
2	Golden Lasa	31.3	29.0	29.8	30.0	+ 4.3
3	Ariwa	30,3	34.0	28.5	30.9	+ 5.2
4	Goldrush	36.8	24.7	28.8	30.1	+ 4.4
5	Enterprise	36.5	38.2	28.5	34.4	+ 8.7
6	Inedit	35.6	28.7	30.3	31.5	+ 5.8
7	Iris	46.8	38.2	48.3	44.4	+ 18.7
8	Luca	39.5	25.2	24.5	29.7	+ 4.0
9	Real	38.8	35.5	29.3	34.5	+ 8.8
10	Rebra	28.0	25.7	30.6	28.1	+ 2.4
11	Redix	29.3	29.5	28.3	29.0	+ 3.3
12	Remar	43.5	38.5	40.3	40.8	+ 15.1
13	Saturn	32.8	37.2	29.3	33.1	+ 7.4
14	Voinicel	33.6	28.5	29.3	30.5	+ 4.8

In the year 2016 (10th year after planting), the largest production was recorded in the varieties 'Golden Lasa', 'Real', 'Rebra', 'Saturn', 'Voinicel', 'Inedit', 'Iris', 'Remar' ranging from 29.3 to 48.3 t/ha. In the other varieties, yields ranging from 24.5 to 28.8 t/ha were recorded, compared to the variety Jonathan, which was registered in 10th year of planting 24.3 t/ha.

Analysing the average of the yields obtained in the three years of study, the yields of the varieties: 'Real' (34.5 t/ha), 'Saturn' (33.1 t/ha), 'Enterprise' (34.4 t/ha), 'Remar' (40.8 t/ha) and 'Iris' (44.4 t/ha). Most varieties have produced over 30 t/ha, being included as branded varieties with high production potential.

Production quality

Apples for fresh consumption or for processing must be healthy, ripen for commercial or consumption purposes and to have organoleptic properties specific to the variety. In order to sell apples at higher prices, they must be at market-standardized levels and maintain their quality after harvesting and during storage and delivery time.

The quality of the fruits is a genetic trait influenced by the variety, the degree of maturation, the action of the environmental factors, as well as of the technological factors. The effects of the interaction of these factors materialize by obtaining fruits with special qualities or they can be negatively influenced.

Low-vigour vegetative rootstocks significantly contribute to the enhancement of apple quality if the varieties grown under appropriate ecological conditions and some technological performance measures are applied.

Fruit Quality Parameters

Average weight and size are important elements in assessing the commercial quality of fruits. These are characteristics of the variety and can be influenced to a greater or lesser extent by the amount of production, the age of the tree, the applied crop technology and the climatic conditions of the year.

The interest in obtaining large fruit with high commercial value is one of the major objectives in the pomological appreciation of the variety. It is known that in European standards, apple size is far superior to that obtained in Romania. Under these circumstances, we must be demanding to choose varieties to promote them in commercial crops and to apply appropriate technology to achieve this goal.

The size of the fruit, combined with an intense and uniform colour (red or golden type) and a symmetrical shape, gives the fruit the appearance and attractiveness required. The study of apple varieties with genetic resistance to diseases grown in high density system reveals a genetic variability in fruit size. A fruit size variability among the varieties studied was found.

It is appreciated that an average fruit size of 170 - 180 g is appropriate for a modern apple variety. Most apple varieties in the study have a fruit size that corresponds to and competes with modern varieties, to the extent that varieties of genetic scab resistance grown in a high density system are promoted in culture in order to obtain some organic production, increasingly demanded by consumers.

The size of the fruit in the 9th year after planting had fairly large amplitude, from 140 g to 'Ariwa', 'Inedit', 'Saturn', to 180 g in the 'Real' variety. Fruit over 160 g were obtained in the varieties 'Enterprise', 'Luca', 'Rebra', 'Redix', 'Remar' and smaller in the varieties 'Goldrush', 'Inedit', 'Iris', 'Ariwa', 'Golden Lasa', 'Saturn' and 'Voinicel', with 140 - 155 g; the differences are due to the genetic factors, respectively the varieties (Table 3).

Table 3: Fruits quality parameters, from apple scab resistant varieties at the harvest date (2015 - 2016)

No	Variety	Fruit weight (g)		Flesh firmness (kgf/cm^2)		Dry substance (%)	
		2015	2016	2015	2016	2015	2016
1	Ionathan	145	148	7.6	8.2	14.2	14.6
2	Golden Lasa	145	165	10.6	10.2	13.8	15.8
3	Ariwa	140	155	10.2	10.5	13.8	15.2
4	Goldrush	145	148	11.4	10.8	14.1	15.0
5	Enterprise	175	165	9.8	10.7	14.0	15.9
6	Inedit	140	158	7.4	8.2	14.2	16.0
7	Iris	145	153	7.8	7.2	13.8	13.6
8	Luca	160	162	10.8	8.7	15.2	15.7
9	Real	180	175	9.6	10.2	13.5	13.0
10	Rebra/M9	170	171	9.1	8.1	14.1	13.4
11	Redix	175	165	10.2	8.6	14.1	15.5
12	Remar	165	172	8.8	8.6	14.0	13.8
13	Saturn	140	160	9.4	9.0	15.4	15.5
14	Voinicel	145	155	7.0	7.5	14.2	14.7

In the 10th leaf, the fruit biomass oscillated from 148 grams at the 'Goldrush' variety (figure 2) and 175 grams at the 'Real' variety. Fruit with biomass over 160 g was obtained in most of the varieties with genetic resistance to the diseases studied: 'Golden Lasa', 'Enterprise', 'Redix' with 165 g, 'Rebra', 'Remar', 'Real' with biomass fruits ranging from 171 to 175 g. The smallest fruits were recorded in the 'Goldrush', 'Iris' and 'Voinicel' varieties with biomass ranging from 148 to 155g.

Figure 2. 'Goldrush' fruit size in overloaded trees

The average value of fruit variety at the level of the variety shows that the varieties 'Enterprise', 'Luca', 'Real', 'Rebra', 'Redix' and 'Remar' have the potential to ensure the large size of the fruit to compete on the market, and the other varieties fall into the middle fruit size group.

The firmness of the fruit pulp is considered to be appropriate when it is compact, crisp or fine, with uniform colouring. A poor firmness is considered to be inappropriate, and is generally found in over-ripen fruits. Also, a rough, coarse consistency with glassy areas is considered negative.

Under the conditions of 2015, the firmness of the fruit pulp at harvest time ranged from 7.0 kgf/cm^2 to the 'Voinicel' variety and 11.4 kgf/cm^2 in the 'Goldrush' variety.

From the data recorded in 2015, it appears that there are varieties producing firm fruit such as: 'Goldrush', 'Ariwa', 'Golden Lasa', 'Enterprise', 'Luca', 'Real', 'Rebra', 'Saturn', 'Redix' and 'Remar', with average penetration resistance of 8.60 kgf/cm^2 and 11.4 kgf/cm^2 and varieties such as 'Voinicel', 'Inedit', 'Iris' and 'Jonathan' as control, whose average fruit penetration resistance is much lower than 7 - 7.8 kgf/cm^2.

In 2016 the firmness of the pulp was between 7.2 kgf/cm^2 in the 'Iris' variety and 10.8 kgf/cm^2 in the 'Goldrush' variety.

The dry matter content of apple varieties with genetic resistance to disease in the years 2015-2016 was 13.0 - 13.5% for ‘Real’ apple variety, being the earliest variety with a dry matter content of 13,4 - 16,0%. For the other varieties, dry matter content accumulated according to the variety and the ripening period of the fruits.

Varietal conveyer of scab apple varieties

Studies and research undertaken at RDSFG Voineşti presents for apple growers, especially for those who promote modern apple culture systems, a group of varieties with different maturation ages covering a long period of consumption with apples of resistant varieties.

Now, the basic range includes the varieties with genetic resistance to diseases: ‘Romus1’, ‘Romus 3’, ‘Romus 4’, ‘Prima’, ‘Pionier’, ‘Voinea’, ‘Ciprian’, ‘Florina’ which are propagated in the fruit trees nurseries in the country. Some of the varieties mentioned, even if they currently meet the requirements of fruit resistance, productivity and quality, can be replaced as new varieties become more valuable, both in terms of production and quality of fruit.

The apple varieties that have been studied can cover much better the consumer season, along with some genetically scab resistant varieties already known and appreciated on the market by consumers.

Table 4 indicate scab apple varieties that were the subject of the study during the period 2014 - 2016 and the way they fit among the valuable varieties with genetic resistance to diseases from the current apple assortment.

Most of the varieties presented are suitable for growing in high-density orchards that will represent future orchards for apple growers.

Depending on the period of maturation and consumption of fruits, the apple varieties studied, which have been distinguished by genetic resistance to disease, productivity and quality of fruit, fall differently in the variety conveyor for Dâmboviţa fruit area.

Thus, according to the varieties of apple ‘Romus 1’, ‘Romus 3’, ‘Romus 4’, ‘Irisem’, the ‘Real’ variety can be introduced, thanks to appetizing fruits, ripen in the last decade of August - first decade of September.

The varieties of apple such as ‘Saturn’, ‘Remar’, ‘Golden Lasa’ fall between the varieties ‘Voinea’ and ‘Pionier’ with the goal of replacing ‘Voinea’ variety by ‘Remar’ because of his superior qualities, taste and fruit coloring.

Table 4: The apple varieties consumption period, in the frame of the scab resistant apple varietal conveyer, cultivated in the Voinesti area

Variety	Month/decade																										
	VII			VIII			IX			X			XI			XII			I			II			III		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Romus1(Vf)		•	•																								
Romus3(Vf)				•	•																						
Romus4(Vf)						•	•																				
Irisem (Vf)						•	•																				
Real (Vf)							•	•	•	•	•	•															
Prima (Vf)							•	•	•	•	•	•															
Voinea (Vf)								•	•	•	•	•															
Saturn (Vf)								•	•	•	•	•															
Remar (Vf)								•	•	•	•	•															
Golden Lasa (Vf)								•	•	•	•	•															
Pionier (Vf)								•	•	•	•	•	•	•	•												
Voinicel (Vf)									•	•	•	•	•	•	•												
Iris (Vf)									•	•	•	•	•	•	•												
Ciprian (Vf)								•	•	•	•	•	•	•	•												
Ariwa (Vf)									•	•	•	•	•	•	•	•	•	•	•	•							
Luca (Vf)									•	•	•	•	•	•	•	•	•	•	•	•							
Rebra (Vf)									•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•			
Redix (Vf)									•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•			
Inedit (Vf)										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Enterprise (Vf)										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Florina (Vf)										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Goldrush (Vf)										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

Also for autumn fresh consumption, the ‘Iris’ and ‘Voinicel’ varieties can be up-scaled, and multiplied along with the ‘Pionier’ variety, which should be replaced.
The ‘Ariwa’, ‘Luca’, ‘Rebra’ and ‘Redix’ varieties fill up the consumption period that extends until January and February.
The varieties ‘Inedit’, ‘Enterprise’ and ‘Goldrush’ exceed Florina's consumption period.

Economic and environmental impact by promoting scab resistant apple varieties

Apple's cultivar assortment is very dynamic due to a competitive market. Is very important to stay economic efficient and therefore, total or partial elimination of the fungicide treatments has a significant role in it.
It can be appreciated that the orientation towards apple varieties with genetic resistance to diseases will gradually be imposed also in high density orchards, not only for the economic efficiency, but also for obtaining ecological productions.
By promoting the cultivation of apple varieties with genetic resistance to diseases adapted to the ecological conditions in our country, we increase the quantity of apples on the market with low pesticide levels, with beneficial influences on the consumers and the environment too.
Due to the reduction of the number of phytosanitary treatments and the quantities of pesticides, the costs are reduced by more than 55%.
The research and development center for fruit growing in Voinesti has been and remains the promoter of the disease-resistant assortment and the high-density apple-tree system in our country.
The economic and environmental effects are highlighted by the costs of phytosanitary treatments per one hectare of orchard.
From the data presented in Table 5, it results that between the two cultivated varieties there are significant differences in the total number of warnings requested during the vegetation period, the quantities of pesticides, the consumption of diesel fuel and the related costs.
Thus, in the orchard with sensitive varieties in the years 2014 - 2016 it was performed 14 sprays; while in the orchard with resistant varieties only 7. The savings made in the orchard with resistant varieties, by the elimination of fungicides in the proportion of 90% and 81% reduction in insecticides and acaricides, represents 66% of susceptible varieties, which means that in orchards with resistant varieties apply 50% fewer sprays and their value is 2 times lower than orchards with a classically sensitive range. Diesel fuel consumption is reduced by 53%.

Table 5. Economic efficiency of some scab resistant apples varieties vs susceptible varieties (2014 - 2016)

Item	Susceptible varieties	Scab resistant varieties	Economic effect (%)
No of sprays	14	7	50
Insect-fungicide consumption (kg,l):	122	54	56
- fungicide (kg,l)	63	6	90
- insecticide – acaricide (kg,l)	59	48	81
Costs (ron):	11808	4464	62
- phytosanitary products	8400	2856	66
- labour	1200	600	50
- mechanic works	2208	1008	54
Diesel consumption (l)	90	42	53
- value (lei)	540	252	53

In addition to the beneficial economic effects, we must add pollution reduction, faster recovery of predators and natural parasites, and maintaining the quality standard of fruit.

CONCLUSIONS

Depending on the diameter of the trunk of the trees, the scab resistant varieties were grouped as follows:

- vigorous varieties: ‘Luca’, ‘Golden Lasa’, ‘Enterprise’, ‘Rebra’, ‘Redix’, ‘Remar’;
- medium-vigorous varieties: ‘Iris’, ‘Inedit’, ‘Voinicel’, ‘Real’, ‘Ariwa’;
- small vigorous: ‘Saturn’, ‘Goldrush’.

The volume of the tree crown recorded in the 10^{th} year since planting indicate ‘Iris’, ‘Goldrush’ and ‘Inedit’ as less vigorous varieties than ‘Enterprise’ variety.
The volume of crown calculated per unit area oscillated between 5,100 cubic meters/ha for ‘Goldrush’ and ‘Inedit’ varieties, up to 8,250 mc/ha in the ‘Enterprise’ variety.

Varieties with small vegetative growth are suitable for expansion in high-density orchards at distances of 4 x 1 m at a density of 2,500 trees / ha.

The highest yield was achieved by ‘Real’ ‘Saturn’, ‘Enterprise’, ‘Remar’ and ‘Iris’

The size of the fruit was influenced by the size of the crop load with fairly large amplitude, from 148 g (‘Goldrush’) to 175 g (‘Real’ variety).

‘Goldrush’, ‘Ariwa’, ‘Golden Lasa’, ‘Enterprise’, ‘Luca’, ‘Real’, ‘Rebra’, ‘Saturn’, ‘Redix’ and ‘Remar’ varieties produce firm fruits with average penetration resistance between 8.60 and 10.8 kgf/cm^2.

The lowest content in dry substance was recorded in the ‘Real’ variety.

The high-density apple system with genetically resistant varieties is recommended for fruit-growing areas in our country due to the high economic efficiency and continuously need for better cultivars.

The elimination of fungicidal products and use of highly selective insecticides, correlated with the quality and productivity of new resistant varieties, support the environmental effects.

REFERENCES

Comănescu D.N, 2002. Cercetări privind sistemul de mare densitate la măr, în scopul obţinerii de producţii adaptate la cerinţele de comercializare, Teză de doctorat.

Comănescu D., Petre G., Petre V., 2012, The behaviour of some apple tree varieties with genetic disease-resistance in the high density system. Scientific Papers. Series B. Horticulture, Vol. LVI, 63-68.

Petre Gh., Petre V., Neagu I.O., 2005. Particularităţile de creştere şi rodire şi efecte ale unor secvenţe tehnologice specifice soiurilor de măr cu rezistenţă genetică la boli, Lucrări ştiinţifice ICPP Piteşti-Mărăcineni, Vol.XXII, Ed. Pământul, Piteşti, 157 – 163.

Petre Gh., Andreieş N., Petre Valeria, 2005. Tehnologia obţinerii unor producţii de mere competitive, Ed. Pildner, Târgovişte.

Petre V., Petre G., Asănică A. 2015, Research on the use of some apple genitors in the breeding process for genetic resistance to disease and fruit quality, Scientific Papers. Series B, Horticulture, Volume LIX, 75-80.

Petre G., Comănescu D. N., Petre V. 2014, Peculiarities of growth and fruitfulness of apple cultivars with genetic resistance to diseases grown under high density system. Scientific Papers. Series B, Horticulture, Volume LVIII, 75-80.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE IMPROVEMENT OF THE ROMANIAN APPLE ASSORTMENT HERITAGE WITH NEW VARIETIES WITH GENETIC RESISTANCE TO DISEASE - REVIDAR, CEZAR AND VALERY

Valeria PETRE[1], Gheorghe PETRE[1], Adrian ASĂNICĂ[2]

[1]Research and Development Station for Fruit Growing Voinesti, 387 Main Street, 137525, Dambovita, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: statiuneavoinesti@gmail.com

Abstract

Between 2014-2016, at the Research and Development Station for Fruit Growing Voinesti it was evaluated the performance of three brand new scab apple varieties: 'Valery', 'Cezar' and 'Revidar', all patented by ISTIS in 2016. The growth vigor of trees in the 9th year, grafted on the M9 rootstock, is well defined by the trunk circumference, which records between 14.9 cm in the 'Caesar' variety and 16.5 cm in the 'Valery' variety. The crown volume calculated at the surface unit and for a density of 2,857 trees/ha was of 8,200 mc/ha for the 'Caesar' and 'Revidar' and 8,900 mc/ha for the 'Valery' variety. Productivity of trees in the ages of 7-9 years was between 35-40 t/ha in the 'Caesar' and 'Valery' varieties and 28.30 t/ha at the 'Revidar' variety. The fruit weight ranged from 160 to 190 g, smaller fruits being recorded in the 'Revidar' variety and higher in the 'Caesar' and 'Valery' varieties, which correspond to market requirements. The Remarkable quality of the varieties fruits recommends them for an increasingly demanding fruit market, meeting the current quality requirements and consumer's needs.

Key words: *Vf resistance, yield, fruit quality, variety.*

INTRODUCTION

Apple's assortment has seen a significant change in the past decades, promoting varieties that primarily target the producer's requirements, sensitive to economic efficiency, high production potential, fruit appearance, ripening time, etc., as well as consumer tastes. These requirements are satisfied by expanding the cultivars of apple varieties with genetic resistance to diseases, which for the new plantations are linked of the efficient economic technology, with immediate effect by the total or partial elimination of the fungicide treatments (Petre V., 2009, 2014).

In the promotion of varieties of apple with genetic resistance to diseases, an important role was played by the Research and Development Station for Fruit Growing Voinesti, either by own creation or by studying foreign varieties, managing to greatly change the vision of the fruit farmers and the gradual change of the assortment.

The continuous breeding process has allowed the creation and patenting of 18 varieties of apple with genetic resistance to diseases (CociuV. et al, 1999), among them 'Valery', 'Cezar' and 'Revidar', were approved in 2016, valuable varieties which will surely meet the growing demands of consumers.

MATERIALS AND METHODS

The complex genetic base existing at the Research and Development Station for Fruit Growing Voinesti, consisting of selection fields, hybrid nursery and competition microcultures, was the main source of selection of valuable apple tree elites and registered with ISTIS for testing for new patenting varieties.

Elite apple H 1/16-90, H 1/78-90, H 4/ 37-04, existing in the microculture set up in 2009 for compete, corresponded in terms of fruit productivity and quality, being registered at ISTIS To be tested for approval from 2014, it being reviewed for 2 years (2014-2015) and analysed according to the DUS and VAT test criteria and techniques required for approval. These became varieties from 2016 under the names 'Revidar', 'Caesar' and 'Valery'.

In order to highlight the performance traits of the three varieties still existing in the competition microculture set up in 2009, the crop technology was applied correctly in order not to affect the production capacity and the quality of the fruits.

The researches carried out during the period 2014-1016 highlight the growth and fruiting potential of new varieties of apple-resistant apple, ‘Revidar’, ‘Caesar ‘and ‘Valery’, where the trees were planted at a distance of 3.5 x 1 m (2,857 trees / ha), grafted on the M9 rootstock, with the spindle shape of a crown.

During the study years, observations and determinations have been made regarding the growth in trunk thickness, tree crown size, production record and fruit quality, by their biomass and dry matter content.

The orchard soil is brown eumesobasic, slightly pseudogley, with a acidic pH (5.7 - 5.9), the humus content is medium to surface (2.0-2.9%), medium supplied with nitrogen, poorly supplied in phosphorus and potassium.

The climatic conditions were favourable for growing and fruiting of trees, characterized by an average annual temperature higher than 1.0°C, normal for the area of 8.8°C, with an annual rainfall of 755 mm.

In the orchard, the soil was maintained as permanent grass cover and weedy clean interval on the row of trees. To control pests, 6 to 8 treatments were applied with insecticides only. The other works were executed according to the technology specific to the high-density orchards.

RESULTS AND DISCUSSIONS

In the process of developing performances in fruit production, varieties must be promoted with a genetic basis that allows:

- increasing the production potential and the quality of the fruit;
- natural increase of resistance to diseases and pests in order to protect the environment;
- suitability in the application of high performance technologies.

An essential condition is compliance with the crop technology in order not to affect the production capacity of the variety and the quality of production. Proper application of technologies preserves or improves varieties' performance.

Obtaining varieties of apple is a long-lasting activity and a great deal of complexity, especially when it comes to obtaining varieties of genetic resistance to disease, irrespective of the research method used.

A new variety, in addition to the productivity, superior fruit quality, genetic resistance to diseases, depending on the area of culture, has to meet other attributes that are added to the essential conditions, namely:

- degree of adaptability to climatic conditions;
- destination of the production, depending on the degree of knowledge of the variety;
- market requirements of the production obtained;
- safety of the production and delivery source of the fruit propagating material;
- economic efficiency of cultural technology.

In the competition microculture, an agrotechnical scheme was applied to the varieties of genetically resistant apple cultivars, including varieties patented in 2016 (mowing the grass between the rows, herbicidation along the row of trees, phytosanitary treatments, only insecticide) and monitoring the vegetation condition of the biological material.

In the 9 years of vegetation, apple elites / apple varieties approved in 2016, ‘Valery’, ‘Cezar’ and ‘Revidar’ have grown properly, fruiting since the 3rd leaf, proving superior quantitative and qualitative production, resistance to scab and mildew.

In order to highlight the characteristics of apple varieties approved in 2016, the growing and fruiting traits recorded during the exam period, namely 2014 - 1016, are forward reproduced.

The vigour of trees in quantitative terms is given by the volume of vegetative growth accumulated annually, expressed by the size of the trunk, the height and size of the tree crown, these being determined by the vigour of the variety, the stable factor being the rootstock.

Growth vigour in tree of nine years old, when the growth potential is well defined, indicates that there are significant differences in the growth in trunk thickness, the height and crown dimensions between the apple varieties with genetic resistance to the diseases under study (Table 1).

Table 1. Growing and fruiting particularities of the new varieties with genetic resistance to diseases patented in 2016

Item	Variety			
	‘Valery’	‘Cezar’	‘Revidar’	Jonathan (control)
I. Growth vigour				
Trunk circumference - cm	16.5	14.9	15.0	14.2
Trees height - cm	290	270	270	280
Fruit tree fence thickness - cm	130	130	130	130
Crown volume - mc/ha	8,900	8,200	8,200	8,500
II. Fruiting				
Phenophases				
Start of blossom	24 – 29.04	22 – 28.04	22 – 28.04	20 – 26.04
End of blossom	01 – 06.05	30.04 – 05.05	30.04 – 05.05	28.04 – 03.05
Blooming time	7 – 9	7 – 9	7 – 9	8 – 9
Date of the fruit ripening	25.09 – 01.10	15 – 20.09	25 – 31.08	20.09 – 30.09
Consumption period	oct. - martie	oct. – decembr.	septembrie	oct. – ianuarie
Storability (days)	145 – 150	80 – 86	30 – 35	125 – 135
Yield (t/ha)	35 – 40	35 – 40	28 – 30	28 – 30
Production quality				
Fruit weight (g)	185	190	160	155
Dry substance content (%)	16.5	13.8	13.0	13.5

The trunk circumference of the three varieties of apple, grafted on the M9 rootstock, recorded values between 14.9 cm in the ‘Caesar’ variety and 16.5 cm in the ‘Valery’ variety, compared to 14.2 cm as recorded to the variety ‘Jonathan’ assigned as a control.

The volume of the crown provides the structure for supporting the branches, the leaves and the fruits and ranges according to the size of the trees. The height of the trees in year 9 records values between 270 cm and 290 cm comparing to the ‘Jonathan’ variety where the height of the trees was of 280 cm. The thickness of the fruit tree fence has values corresponding to the three varieties of apple taken in the study, including the 130 cm of the control variety.

The volume of the crown calculated at the surface unit at the planting density of 2,857 trees / ha was of 8,200 mc / ha for the ‘Caesar’ and ‘Revidar’ varieties and 8,900 mc / ha for the ‘Valery’ variety, with the medium values at the ‘Jonathan’ variety as a control with 8,500 mc / ha.

Based on vigour values, the present varieties studied have been included into middle-strength varieties (‘Valery’) and small-medium vigorous varieties (‘Caesar’, ‘Revidar’).

For the evaluation of the rhythm of the fructification phenophases, the data related to: the beginning and the end of the flowering, its duration, but also the maturation period of the fruits were recorded.

From the dates recorded between 2014 and 2016, the first flowers were opened in ‘Caesar’ and ‘Revidar’ varieties from April 22 to 28, followed by the ‘Valery’ variety between 24 and 29.04. The end of the bloom took place between 30.04 and 06.05 so that the three varieties studied are enclosed into varieties with a medium flowering period and a flowering period of 7 to 9 days, depending on the evolution of climatic conditions.

The varieties taken in the study overlapped totally or partially during flowering, allowing mutual pollination.

Fruit maturation took place between 25th and 31st of August in the ‘Revidar’ variety, being considered as summer variety, 15-20.09 in the ‘Caesar’ variety and 25.09-01.10 in the ‘Valery’ variety, which has a longer storage of the fruits.

The duration of fruit storage was 30-35 days for the ‘Revidar’ variety, 80-86 days for the ‘Caesar’ variety and 145-150 days for the ‘Valery’ variety, compared to the ‘Jonathan’ variety as control, where the storage duration of the apples in the cooling space is 125 - 135 days.

One of the priority objectives of the study is the evaluation of the production capacity, being the most important characteristic in promoting of the varieties for the establishment of the new commercial plantations. The high productive potential, associated with superior fruit quality, expresses to the highest degree the capacity of apple varieties with genetic resistance to diseases, taken in the study to assimilate and capitalize the ecological conditions of the area in which they are grown.

Productivity of apple varieties is a complex attribute, genetically determined by the hereditary base from which it originates, but is influenced by the interaction between the variety and the climatic conditions of the area of culture. Other factors contributing to the shaping of this attribute are related to the yielding precocity, the type of fructification, applied technology, disease resistance, grafting and pollination compatibility, planting density and rootstock used.

The production potential at 7 to 9 trees year old in the experimented varieties of apple cultivated in a high-density system was between 35 to 40 t / ha for the 'Caesar' and 'Valery' varieties and of 28 to 30 tons / ha in the 'Revidar' variety.

The quality of the fruits expressed in their biomass was between 160 g and 190 g, smaller fruits registered in the 'Revidar' variety and higher in the 'Caesar' and 'Valery' varieties. Dry matter content ranged between 13% and 16.5%.

Apple varieties with genetic resistance to diseases, patented in 2016, have other valuable features, as follows:

- 'Valery' (sin. H 4/37 - 04) - fruits are conic-truncated, yellow-orange on the sunny side, with a yellowish-yellow pulp, crisp at harvest, with a sweet and very good taste (Figure 1). It is resistant to scab and poorly attacked by *Podosphaera leocotricha*. It is characterized by precocity, outstanding fruit quality and constant production.

Figure 1. 'Valery' fruits

- 'Cezar' (sin H 1/79 - 90), present conical-globular fruits, covered with red on almost the entire surface (Figure 2). White, sweet, slightly acidified pulp is very tasty. It is resistant to *Venturia inaequalis* and *Podosphaera leocotricha*. It is distinguished by small-medium vigour, precocity, fruit quality and constant production.

Figure 2. 'Cezar' fruits

- 'Revidar' (sin H 1/16 - 90), have conical-shaped fruits, covered in red on 2/3 of the surface (Figure 3). The pulp is white, acidified and juicy, with good taste. It is resistant to scab and mildew. It is distinguished by medium-small growth, precocity, productivity and constant yielding trait.

Figure 3. 'Revidar' fruits

The studies and researches undertaken at RDSFG Voineşti present for apple growers a group of varieties with different ripening range covering a long period of consumption with apples from genetically resistant varieties.

Nowadays, the basic assortment includes varieties with genetic resistance to diseases such as: 'Romus 1', 'Romus 3', 'Romus 4', ‘Prima’, ‘Pionier’, ‘Voinea’, ‘Ciprian’, ‘Florina’, which are propagated in the Romanian fruit nurseries. Some of the varieties mentioned, even if they currently meet the requirements in terms of fruit resistance, productivity and quality, they can be replaced by new varieties more valuable in this respect.

Table 2 displays apple varieties with genetic resistance to diseases, ‘Revidar’, ‘Caesar’ and ‘Valery’, which were the subject of the study between varieties of apple with genetic resistance to diseases from the current assortment of apple.

Table 2. Consumption time and position of brand new varieties inside the assortment chart of the scab resistant varieties in the Voineşti area

Variety	Month																										
	VII			*VIII*			*IX*			*X*			*XI*			*XII*			*I*			*II*			*III*		
	1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	***2***	***3***
Romus 1 (Vf)		•	•																								
Romus 2 (Vf)		•	•																								
Romus 3 (Vf)				•	•																						
Irisem(Vf)						•	•																				
Real (Vf)						•	•																				
Prima (Vf)						•	•	•																			
Revidar (Vf)						•	•	•	•																		
Remar (Vf)								•	•	•	•	•															
Iris (Vf)								•	•	•	•	•															
Voinea (Vf)									•	•	•	•															
Voinicel (Vf)									•	•	•	•	•	•	•												
Pionier (Vf)									•	•	•	•	•	•	•												
Cezar (Vf)										•	•	•	•	•	•	•	•	•									
Inedit (Vf)										•	•	•	•	•	•	•	•	•	•	•	•	•	•				
Generos										•	•	•	•	•	•	•	•	•	•	•	•	•	•				
Redix (Vf)										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•			
Valery (Vf)										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•		
Florina (Vf)										•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

Depending on the period of maturation and consumption of fruits, the apple varieties studied, which have been distinguished by genetic resistance to disease, productivity and quality of fruit, fall differently in the variety conveyor for Dâmboviţa fruit basin.

Therefore, after the apple varieties as ‘Romus 1’, ‘Romus 3’, ‘Romus 4’, it can be introduced ‘Irisem’ and ‘Real’, varieties with ripening time in the last decade of August to first decade of September. After ‘Prima’ variety, the apple variety ‘Revidar’, which has a consumption period from 25 to 31 August until 1 October is very welcomed.

‘Remar’ apple variety is situated between the varieties ‘Voinea’ and ‘Pionier’, with the perspective of replacing ‘Voinea’ by the ‘Remar’ variety, because it has some superior qualities, both for taste and for fruit coloring.

For autumn season, the ‘Iris’ and ‘Voinicel’ varieties represent also the alternative for ‘Pionier’ variety.

After the ‘Pionier’ variety, the ‘Caesar’ variety filling gaps with the fruits harvested between September 15 - 20 and consumed until the end of December.

‘Redix’ variety completes a period of consumption that extends until December to January.

‘Inedit’ variety exceeds ‘Redix’ consumption period, closer to that of the ‘Florina’ variety. During the ‘Florina’ variety season, the ‘Valery’ variety arises, with Golden delicious fruit type.

The orientation towards apple varieties with genetic resistance to diseases in Romania will gradually be imposed not only as a result of economic efficiency, but also for the fact that they are the main factor in obtaining organic production.

CONCLUSIONS

- The new varieties of apple ‘Valery’, ‘Cezar’ and ‘Revidar’, patented in 2016, meet the requirements of the producers, oriented to economic efficiency, high production potential, quality fruits, and consumers demands.
- The growth vigour of the trees in the 9^{th} year after planting, represented by the circumference of the trunk and trees height, rank ‘Valery’ variety as more vigorous than “Revidar” and “Cezar” which are similar in vigour.
- The volume of the crown calculated at the surface unit, at the density of 2,857 trees / ha, oscillated between 8,200 mc / ha in the ‘Caesar’ and ‘Revidar‘ varieties, and 8,900 mc / ha in the ‘Valery’ variety.
- The highest production potential was achieved in the 7^{th} - 9^{th} years by ‘Caesar’ and ‘Valery’ varieties with yields of 35-40 t / ha and 28-30 t / ha by the ‘Revidar’ variety.
- Apples varieties created and patented by RDSFG Voineşti in 2016 cover a large period of the consumption season alongside other varieties with genetic resistance to diseases already known and appreciated on the market by consumers, completing the conveyer recommended for Dambovita area.
- By promoting the varieties of apple with genetic resistance to diseases, there are beneficial economic outcomes for producers, environmental protection and apple production with low pesticide residues.

REFERENCES

Cociu V., Botu I., Şerboiu L., 1999. Progrese în ameliorarea plantelor horticole din România. Vol.I., Pomicultura. Edit. Ceres, Bucureşti.

Collectively by authors, 2000. Staţiunea de cercetare şi producţie pomicolă Voineşti la aniversarea a 50 de ani de cercetare ştiinţifică şi dezvoltare (1950 -2000). Ed. Domino, Târgovişte.

Collectively by authors, 2014. Genotipuri pomicole tolerante la stress termic, hidric şi biotic, pretabile sistemelor tehnologice specifice agriculturii durabile. Subcap. 4.1. Ameliorarea mărului şi părului la SCDP Voineşti. Ghid elaborat în cadrul proiectului sectorial ADER 1.1.9., finanţat de MADR.

Petre V., Petre Gh., 2014. Contributions regarding the apple trees genetic variability increase in the process of obtaining improving biological material. Scientific papers. Series B. Horticulture., Vol LVIII, 71-74.

Petre V., 2009. Tehnica obţinerii soiurilor de măr cu rezistenţă genetică la boli prin mutageneză. Ed. Moroşan, Bucureşti.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECT OF AUXIGER GROWTH REGULATOR ON FRUITS DEVELOPMENT, PRODUCTION AND CHRACING INDEX OF 'REGINA' CHERRY VARIETY

Ananie PEŞTEANU, Valerian BĂLAN, Igor IVANOV

State Agrarian University of Moldova, 44 Mircesti Street, MD-2049, Chisinau, Republic of Moldova

Corresponding author email: a.pesteanu@uasm.md

Abstract

Fruit size and yield can be improved with the application of growth regulators, such as synthetic auxins and may be effective in enhancing fruit growth, when applied during the second stage of fruit development. The aim was to evaluate the influence of Auxiger growth regulator on average weight of fruits, fruit production, fruit size, period of maturation and cracking index. The study subject of the experience was 'Regina' cherry variety, grafted on Gisela 6. The trees were trained as spindle system. The distance of plantation is 4.0 x 2.0 m. The experimental plot it was placed in the orchard „Vindex-Agro" Ltd. founded in 2012 year. The research was conducted during the period of 2016 year. To study average weight of fruits, fruit production, fruit size, period of maturation and cracking index were experimented the following variants of treatment: 1.Control – without treatment; 2. Auxiger, 0.5 l/ha; 3. Auxiger, 0.7 l/ha. Active ingredient of Auxiger is NAD – 1.5 g/l + ANA – 0.6 g/l. Growth regulator Auxiger were sprayed one time, during the period of intensive fruit growing, when the fruits diameter was 12-13 mm (26.05.16). During the research, it was studied the average of fruits, tree production and their quality, period of maturation and cracking index. During the analyzed period, it was established that the average weight of fruits, the plantation productivity, fruit size, period of maturation increase when treating with Auxiger growth regulators in dose of 0.7 l/ha and reduced the cracking index when the diameter of the fruits was 12 - 13 mm.

Key words: *growth regulator, fruit size, production, quality, maturation.*

INTRODUCTION

Cherries are the first fresh fruit of the year. Cherry fruits have a significant food value (Asanică, 2012; Cimpoieş, 2002). The food value, as well as other qualities of the fruits, such as size, color and firmness of flesh, are generally the main criteria by which the destination to exploit the cherries is determined (Asanică et al., 2013; Balan, 2015).

Small fruit size is one of the limiting factors in marketing cherry fruit (Sansavini and Lugli, 2005; Whiting and Ophardt, 2005).

As consumers prefer large cherries, fruit size is a very important marketing consideration, and the economic benefits of treatments capable of improving average fruit size are potentially very high.

Several techniques have been used to improve fruit production and fruit size of cherry (Balan, 2012; Budan and Grădinăriu, 2000; Long et al., 2014; Whiting and Lang, 2004). Fruit size can be improved with the application of growth regulators, such as synthetic auxins and gibberellins (Zeman et al., 2013).

Synthetic auxins may be effective in enhancing fruit growth, when applied during the second stage of fruit development (Faust, 1989).

The effectiveness of synthetic auxins in increasing fruit size is affected by the type of auxins, its concentration and the fruit crop. Some synthetic auxins are effective in increasing fruit production and fruit size of sweet cherry (Stern et al., 2007), though others, such as CPA, showed no effect (Zhang and Whiting, 2011).

In the fruit growing practice, the growth regulators are used in small amounts, but their effect is quite striking, if applied in recommended phases in active physiological concentrations, allowing be easily absorbing and transporting to the reaction.

NAA applied alone or in combination 30 - 35 days before the harvest decrease cracking index (Demirsoy and Bilgener, 1998). Pre-harvest spray of NAA has also been reported to reduce the field cracking and cracking index and increase the firmness of two cherry varieties (Anonymous, 1994; Yamamoto et al., 1992).

A combination of auxins gives better results than the application of single compound (Long et al., 2014; Stern et al., 2007; Zeman et al., 2013).

The objective of this study was to evaluate the effect of growth regulator Auxiger (NAD and NAA) on fruit development, fruit size, cracking, maturation, quality and yield in Regina sweet cherry.

MATERIALS AND METHODS

The research was conducted during the year of 2016, in the cherry orchard founded near the village Malaesti, Orhei district, during the spring of 2012 in the „Vindex-Agro" Ltd., with one-year-old trees shaped as a rod. The subject of the experience was 'Regina' cherry variety grafted on rootstock Gisela 6. The crowns it conducted by thin spindle system. The planting distance was 4.0 x 2.0 m.

To establish the influence of Auxiger growth regulator on the plantation production, cherry fruits quality and they cracking were tested the following variants (Table 1).

Table 1. Scheme experiments to determine the effectiveness of Auxiger growth regulator on tree production and their quality

Variants	Active ingredient	Application
Control	-	-
Auxiger, 0.5 l/ha	NAD - 1.5 g/l + NAA - 0.6 g/l	Spraying during the period of intensive fruit growing
Auxiger, 0.7 l/ha		

In the second and third variant the treatment date was 26.05.16, when was registered an intensive cherry growing. Location of plots made into blocks, each variant having four replicates. Each replication has 7 trees. At the border between the rehearsals and experimental plots were left one untreated tree to avoid the duplication of variants or repetitions while performing treatments.

Trees treatment was performed with portable sprinklers in the morning hours. The amount of the solution was 0.8 liters tree, based on the number of trees per unit area and the amount of water recommended of 1000 l/ha.

The number of fruits, the average weight of a fruit, the production from a tree and a unit area were settled during the harvest. The establishment of harvest for each variant was performed by individual weighing of the fruits on 28 trees. The average weight of the fruits was determined by weighing a sample of 1 kg of cherries from each repetition and counting them.

The fruit diameter was determined during the harvesting period using the template recommended for sorting cherries by holes of 26, 28, 30, 32, 34 and 36 mm.

The height of the fruits was determined by the measuring and it is the distance between the base and the top. The large and small diameter of a fruit was measured at the equatorial area. The evaluation of mentioned parameters was carried out using calipers at time of harvest gathering 20 fruits in the row from each repetition.

The average weight of the seed was determined by the method of weighing, an indicator which was obtained as a result of removing the pulp from the seed. The ratio of the seed in the fruit is the ratio between the weight of 20 seeds and the weight of these fruits in each repetition reported in percent.

To have a more real index of cracking of cherry fruits, it resorted to setting cracking index of cracking natural and artificial. Natural cracking index was determined by the counting method at harvest time. After collecting 100 fruits in a row from the tree crown, it was counted the number of cracked fruits, then, using the correlation was established the index of cracking. Theoretically, the cracking index was determined by the method described by Christensen (1972).

Fruit harvesting was carried out in two rounds based on their maturation. The share of fruits harvested in the first half and the second one was determined by the method of weighing and counting on specific trees out of each variant.

The significance of differences men values of investigated parameters was determined by using the LSD test for the likelihood of 0.05.

The soil between the rows consisted of grass silage, and the strips between the trees per row with a width of 1.2 m are worked with the mechanical milling FA 086. The irrigation in the plantation is carried out by drip irrigation. The fertilization system calculated according to soil fertility and scheduled crop.

RESULTS AND DISCUSSIONS

The fruit production is the final index which indicates how all agro-technical measures were performed in the cherry plantation 'Regina' variety.

Investigations conducted proved that the number of fruit in the trees crown included in the research were not different in the studied variants (Table 2). This is explained by the fact that to create identical conditions for fruit development was necessary to leave a constant number of fruits in the trees crown. To maintain this number of fruit in the trees crown after the fall of ovaries in June, the load of fruit was corrected by manual thinning, leaving a number as precisely as possible of fruits.

Table 2. The influence of the Auxiger growth regulator on the amount of fruits, the average weight of fruits and the production of 'Regina' cherries variety

Variants	Number of fruits, pcs/tree	Average weight, g	The production of fruit		In %, compared to control variant
			kg /tree	t/ha	
Control	495	10.07	4.98	6.23	100.0
Auxiger, 0.5 l/ha	491	10.68	5.24	6.55	105.1
Auxiger, 0.7 l/ha	498	10.83	5.39	6.74	108.2
LDS 0.05	18.4	0.33	0.14	0.18	-

The lowest average weight of fruits was recorded in the control variant, without treatment being 10.07 g. Followed, in the ascendant order by the variant treated with Auxiger in dose of 0.5 l/ha with an average fruit weight of 10.68 g and the variant treated with Auxiger in dose of 0.7 l/ha, where the studied index was 10.83 g, or an increase of 0.76 g compared with the control variant. This difference in average weight between control variant and variants 2 and 3 was recorded due to treatment with growth regulator Auxiger.

Analyzing the influence of the dose treatment, it was recorded that with the increase of the dose quantity from 0.5 to 0.7 l/ha, the average fruit weight increased, but not as much as it increased between the control variant and the treated variants. If the difference between the variant treated with Auxiger in dose of 0.5 l/ha and 0.7 l/ha was 0.15 g, then between the control variant and the treated variant with the growth regulator Auxiger in dose of 0.5 l/ha was 0.61g. This results were proven statistically too.

The production of fruits on a tree and a surface unit is in direct correlation with the number of fruits and their average weight. The lowest fruit production was recorded in the control variant being 4.98 kg/tree or 6.23 t/ha.

In the variant treated with Auxiger in dose of 0.5 l/ha, the fruit production was 5.24 kg/tree or 6.55 t/ha, or it increased with 5.1% compared with the control variant.

The highest fruit production was registered in the variant treated with Auxiger in dose of 0.7 l/ha being 5.39 kg/tree or 6.74 t/ha, or and increase with 8.2% compared with the control variant.

Studying the influence of treatment dose on fruit production showed that with increasing the amount of product administered from 0.5 to 0.7 l/ha, the studied index increased, but not as essentially as between control variant and tested variants. If the difference between the treated variant with Auxiger in dose of 0.5 l/ha and 0.7 l/ha was 3.1%, then between the control variant and the variant treated with Auxiger in dose of 0.5 l/ha was 5.1%.

The insignificant difference between the variant treated with Auxiger in dose of 0.5 l/ha and Auxiger 0.7 l/ha was proven statistically too.

Statistical data about the production of fruit from a tree and a unit area showed a statistical difference between the control variant and variants treated with Auxiger.

Currently, in the modern research conducted on cherry plantations in order to increase the average weight of the fruits and their quality parameters (height, width, thickness, seed weight) are widely used treatments with growth regulators from auxin group.

While studying the fruit size 'Regina' cherry variety, we recorded higher values on their large diameter (d_1), and then in descendent order was the height and lastly the small diameter (d_2). If the large diameter during the research was 30.7 - 31.5 mm, then the height index and the small diameter was respectively 28.0 - 29.0 and 27.1 - 28.3 mm (Table 3).

Between the studied variants, the lowest height of a fruit was recorded in control variant, being 28.0 mm. In ascendant order is placed the variant treated with Auxiger in dose of 0.5 l/ha, with the studied index being 28.7 mm. Followed by the variant treated with Auxiger in dose of 0.7 l/ha, where the height of a fruit was

29.0 mm, or it increased with 3.6% compared with the control variant.

Table 3. Influence of Auxiger growth regulator on the quality of cherry fruits of 'Regina' variety

Variants	Size , mm			H/D	Average seed weight, g	% of seed
	Height (h)	Large diameter (d_1)	Small diameter (d_2)			
Control	28.0	30.7	27.1	0.91	0.58	5.7
Auxiger, 0.5 l/ha	28.7	3.3	27.9	0.92	0.59	5.5
Auxiger, 0.7 l/ha	29.0	3.5	28.3	0.92	0.59	5.4

Analyzing the influence of the treatment dose on the fruit height, it was noticed that once the treatment dose increased the height of the fruit increased too. If the difference between the variant treated with Auxiger in dose of 0.5 l/ha and dose of 0.7 l/ha was 1.1%, then between control variant and the variant treated with the Auxiger in dose of 0.5 l/ha was 2.5%.

At harvest time, the smallest value of the large diameter on cherry fruits was recorded in the control variant, being 30.7 mm. When treatment was applied with Auxiger, an increase in the studied index was noticed being 31.3 - 31.5 mm, so it increased with 0.6 - 0.8 mm compared with the control variant. The increase in the treatment dose did not influence significantly the large diameter index on Regina cherry variety. The same thing is valid and for the small diameter perhaps with small deviations between the variants.

The treatments made with Auxiger also influenced on the ratio between the height and the large diameter on the fruits. The smallest value of this ratio was registered in the control variant, being 0.91. In the variants treated with Auxiger, the ratio height/large diameter of fruits was 0.92.

The size of the seed is an important index for the quality of the fruits and productivity. On different varieties of cherries the seed ratio stands around 7.0%, but cherries varieties are quite different (Donica Il et al., 2005).

The conducted researches showed at the average seed weight in the control variant, was the smallest being 0.58g, but when treatment was made with Auxiger, its value was 0.59 g.

The seed ratio in the fruit is influenced by the average seed weight and the average fruit weight. Conducted research highlighted the variants treated with Auxiger where the seed weight was 5.4 to 5.5%. In the control variant, the above index was higher, being 5.7%. Therefore, the treatments made with had a positive influence both on height, width and thickness of the fruit, and also on the fruit and the seed weight.

Effectuated research showed that there is a direct influence between the fruit weight and their diameter. The results from table 4 show that the fruit production obtained in the studied variants differ, registering higher values when treating with the Auxiger growth regulator.

If, in the control variant, the diameter of fruits with 22-26 mm was 24.7%, the fruits with 26-30 mm diameter were 30.1% and the fruits with the diameter larger than 30 mm were 45.2%. Therefore, the fruits with the diameter larger than 26 mm in the control variant were 75.3%.

Table 4. The influence of Auxiger growth regulator on fruits redistribution according to their diameter in the 'Regina' cherry variety

Variants	The share of fruits (%) according to their diameter (mm)		
	22-26	26-30	>30
Control	24.7	30.1	45.2
Auxiger, 0.5 l/ha	14.6	28.7	56.7
Auxiger, 0.7 l/ha	12.8	27.7	59.5

When treatment was made with Auxiger, the cherry fruit quality improved compared to the control variant. When treatment was made with Auxiger growth regulator in dose of 0.5 l/ha, the share of fruits with the diameter 22 - 26 mm decreased in comparison with the control variant being 14.6%, those with diameter 26 - 30 mm were 28.7%. The fruits with a diameter larger than 30 mm increased to 11.5%. This means that the share of fruits with the diameter larger than 26 mm were 85.4% or it increased with 10.1% compared with the control variant.

The same thing was valid and for the variant treated with Auxiger in dose of 0.7 l/ha. The share of fruits with the diameter 22 - 26 mm decreased in comparison with the control variant, being 12.8%, those with diameter 26 - 30 mm were 27.7% and the fruits with a diameter larger than 30 mm - 5.5%. Practically, this variant showed higher values compared with the control variant and the variant treated with Auxiger in dose of 0.5 l/ha.

By studying the influence of the dose treatment on the distribution of cherry fruit by diameter, once the amount of product increased from 0.5 to 0.7 l/ha, the studied index increased too, but not as much as compared with the control variant. If the difference between the fruits with a diameter larger than 2 mm between the variant treated with Auxiger in dose of 0.5 and 0,7 l/ha was 1,%, then between the control variant and the variant treated with Auxiger in dose of 0.5 l/ha was 20.1%.

Cherry fruit cracking is an inherent characteristic of the species and under certain genetic, physiological, chemical conditions can affect up to 90% of the harvest which influences negatively the financial situation of companies (Demirsoy and Bilgener, 1998).

The factors that may promote the phenomenon of cracking of the cherry fruits can be chemical, technological and genetically. They influence on the maturation of the cherry, the intensity of respiration, the capacity to absorb the water at the root and the skin of fruit, and also the osmotic pressure and turgor potential of the mesocarp cells (Yamamoto et al., 1992).

Cherry fruits are more prone to cracking during the period when they move from the yellow - purple color until they become black which is considered the full maturation and they are ready for consume. During the reference period (10-29.06.2016), the quantity of atmospheric precipitation was 85.9 mm.

These rainfalls affected the natural fruit cracking index on Regina cherry variety. The high'est value of the natural fruit cracking index on 'Regina' cherry variety was recorded after precipitation fallen during their maturation were in the control variant was 2.0%. In the variants treated with growth regulators Auxiger, it did not register fruits cracked naturally despite the precipitation fallen during fruit ripening.

To have a more real value of the theoretical fruit cracking index on 'Regina' cherry variety, it was used the method described by Christensen (1972).

Conducted research after two hours of cherries fruits immersion in water, it demonstrated that in the control variant, only one fruit cracked. The number of fruits cracked in the same variant after being immersed in water for four hours was 2 pcs, and after 6 hours - 7 pcs. The obtained results showed that the theoretical index of artificial cracking was 7.2% (Table 5).

In the variants treated with Auxiger, after the fruits were immersed in water for 2 and 4 hours, don't were registered the cracked fruits. If, the period of time that the fruits where in the water increased to 6 hours, it was recorded the quality of cherries improved compared to the control variant. In the variant treated with Auxiger in dose of 0.5 l/ha, the number of fruits artificially cracked was 4 pcs, or it decrease of 5.6% compared with the control variant.

Table 5. The influence of the growth regulator Auxiger on the fruit cracking on cherries of 'Regina' variety

Variants	Index of natural cracking, %	Fruits cracked artificially, psc.			Index of theoretical cracking, %
		After 2 hours	After 4 hours	After 6 hours	
Control	2.0	1	2	7	7.2
Auxiger, 0.5 l/ha	-	-		4	1.6
Auxiger, 0.7 l/ha	-	-	-	3	1.2

The same thing happened and in the variant treated with Auxiger in dose of 0.7 l/ha where the number of cracked fruit artificially was 3 psc, or it decreased by 6.0% compared with the control variant.

Analyzing the influence of the treatment dose on the artificially fruit cracking, it was noticed that once the dose treatment increased for 0.5 to 0.7 l/ha, the studied index didn't change as much as it did in the control variant. If the difference between the artificially cracked fruits in the variant treated with Auxiger in dose of 0.5 and 0.7 l/ha was 0.4%, then between the control variant and the variant treated with Auxiger in dose of 0.5 l/ha was 5.6%.

Optimal harvest time is determined by the fruit capitalization way. In this context, it should be borne in mind the gradual maturation of cherries and that after their separation from the tree no longer occur physiological processes to improve quality, as happens in other species. Therefore, cherry fruits are collected in two phases, during the time when they have the highest food value and good taste. The best harvesting time is determined usually empirically based on experience taking into account the color of the fruit, since there is no other index more accurately. Thus, the cherries are harvested when they got the color typical of the variety, the flesh softens and releases easily from the stalk branch.

Conducted research proved that treatments made with growth regulators Auxiger intensified fruit coloring. ’Regina’ cherry variety is a late maturing variety which requires for the fruits to be collected in two stages.

The most important index is the share of fruits picked in the first and second stage of harvest. The research showed that in the control variant in the first picking stage (27.06.2016) were collected 48.8% of fruits from the trees crown and in the second stage (30.06.206) were picked the rest 51.2% (Table 6).

The treatments performed with the growth regulator Auxiger which is based on active ingredients NAD and NAA increased the share of fruits pick in the first stage of harvest.

Table 6. The influence of Auxiger growth regulator on the maturation of fruits of ’Regina’ cherry variety, %

Variants	Harvest time	
	27.06.2016	30.06.2016
Control	48.8	51.2
Auxiger, 0.5 l/ha	67.5	32.5
Auxiger, 0.7 l/ha	70.4	28.6

When treatments were made with Auxiger in dose of 0.5 l/ha, the share of fruits picked in the first stage of harvest was 67.5% or it increased with 18.7% compared with the control variant. Once, the treatment dose increased to 0.7 l/ha, the studied index increased to 70.4%, which increased by 21.6% compared with the control variant and a 2.9% increase compare with the variant where the treatment dose was 0.5 l/ha.

CONCLUSIONS

Treatments made with Auxiger in dose of 0.7 l/ha increased the average weight of fruits, the plantation productivity, fruit size, period of maturation and reduced the cracking index.

The results presented here, indicate that the effect of the application of plant growth regulator Auxiger in dose of 0.7 l/ha during the intensive cherry growth, when the fruits reach a diameter was 12 - 13 mm, improve the physiological processes of the plant and increase the fruit development, quality and yield of ’Regina’ sweet cherries variety.

REFERENCES

Anonymous , 1994. All about cherry cracking. Tree Fruit Leader, 3: 1-11.

Asanică A., 2012. Cireşul în plantaţiile moderne. Bucureşti.

Asanică A., Petre Gh., Petre V., 2013. Înfiinţarea şi exploatarea livezilor de cireş şi vişin. Bucureşti.

Balan V., 2012. Perspective în cultura cireşului. Pomicultura, Viticultura şi Vinificaţia Moldovei. Chişinău, 2: 7.

Balan V., 2015. Tehnologii pentru intensificarea culturii mărului şi cireşului. Akademos, Chişinău, 3: 82-87.

Budan V., Grădinăriu G., 2000. Cireşul. Iaşi.

Christensen J. V., 1972. Cracking in cherries. I. Fluctuation and rate of water absorption in relation to cracking susceptibility. Tidsskr. Planteavl, 76: 1-5.

Cimpoieş Gh., 2002. Pomicultura specială. Chişinau.

Demirsoy L., Bilgener S., 1998. The effect of preharvest chemical applications on cracking and fruit quality in 0900 Zirat, Lambert and Van sweet cherry varieties. Acta Hort., 468: 663-670.

Donica Il., Ceban E., Rapcea M., Donică A., 2005. Cultura cireşului. Chişinău.

Faust M., 1989. Physiology of Temperate Zone Fruit Trees. Wiley, New York, USA, 169-234.

Long L. E., Lond M., Peşteanu A., Gudumac E., 2014. Producerea cireşelor. Manual tehnologic. Chişinău.

Sansavini S., Lugli S., 2005. Trends in sweet cherry cultivars and breeding in Europe and Asia. Proc. 5th Int. Cherry Symposium. Turkey, (Abstract), 1.

Stern R.A., Flaishman M., Applebau, S., Ben-Arie R., 2007. Effect of synthetic auxins on fruit development of “Bing” cherry (Prunus avium L.). Sci. Hortic. Amsterdam, 114: 275-280.

Yamamoto T., Satoh H., Watanabe S., 1992. The effects of calcium and naphthalene acetic acid Sprays on cracking index and natural rain cracking in sweet cherry fruits. J. Japan. Soc. Hort. Sci., 61: 507-511.

Whiting M.D., Lang G.A., 2004. “Bing” sweet cherry on the dwarfing rootstock Gisela 5: thinning affects tree growth and fruit yield and quality but not net CO2 exchange. J. Am. Soc. Hort. Sci., 129: 407-415.

Whiting M.D., Ophardt, D., 2005. Comparing novel sweet cherry crop load management strategies. HortScience, 40; 1271-1275.

Zeman S., Jemrić T., Čmelik Z., Fruk G., Bujan M., Tompić T. 2013. The effect of climatic conditions on sweet cherry fruit treated with plant growth regulators. Journal of Food, Agriculture and Environment, 11(2): 524-528.

Zhang C., Whiting M., 2011. Pre-harvest foliar application of prohexadione-Ca and gibberellins modify canopy source-sink relations and improve quality and shelf-life of “Bing” sweet cherry. Plant Growth Regul., 65: 145-156.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EMERGING PESTS OF *ZIZIPHUS JUJUBA* CROP IN ROMANIA

Roxana CICEOI[1], Ionela DOBRIN[2], Elena Ştefania MARDARE[1], Elena Diana DICIANU[2], Florin STĂNICĂ[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Laboratory of Diagnosis and Plant Protection of Research Center for Studies of Food Quality and Agricultural Products, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: roxana.ciceoi@gmail.com

Abstract

The jujube crop (Ziziphus jujuba Mill.) is one of the oldest crops in China and in the world. Written indications about its technology (grafting, pruning, pest control etc) are older than 1500 years, when the jujube was one of the top five most important fruits in China, together with peach, apricot, plum and chestnut. Their nutraceutical qualities were highly appreciated, but also the beauty of the tree and the flower perfume, which legends says, made people fall in love. Nowadays, the crop is expanding, both in China and on the other continents, due to the high content of bioactive compounds that can help in lowering the blood pressure, in liver diseases, anaemia and also inhibit the growth of tumor cells etc. In Romania, the species grows sub spontaneous since the antique times and until the present moment no important pests had been reported. Still, giving the climatic changes in the last years, many pests became invasive and enlarge their host plant spectrum. On the USAMV Bucharest experimental field, we monitor the pests incidence and we identify few species that might become a threat: Halyomorpha halys (Stal) (Heteroptera: Pentatomidae), Metcalfa pruinosa Say (Homoptera: Flatidae), Ceratitis capitata (Wiedemann) (Diptera, Tephritidae) and Nezara viridula (L.) (Heteroptera: Pentatomidae). The other potential polyphagous pests, like weevils, fruit borers and moths, made insignificant damages on the fruits, but they should still be under careful observation. Data about the pests' biology, damage and control are given.

***Key words**: Zizipus jujuba, Halyomorpha halys, invasive alien species, polyphagous pests, quarantine pests.*

INTRODUCTION

The jujube fruits are nowadays worldwide appreciated as an outstanding source of biologically active compounds, with high nutraceutical value (Liu et al., 2014, Preeti et Tripathi, 2014). According to Gupta, 2004, jujube, along with date palms and grapes, start to be domesticated on the Indian subcontinent around the year 9000 BC, together with wheat and barley, which were cultivated from the very beginning of agriculture. In China, many written sources date back the jujube fruits 4000 years ago (Li et al., 2007, Liu et al., 2009) while in Romania it seems the tree was brought via the Silk road 2000 years ago, (Stănică, 2009). Jujube had been used both as crude and dried in the Chinese Traditional Medicine, for its antitussive, palliative, analeptic and nutraceutical properties, but also as a food and food flavourant for thousands of years (Li et al., 2007). It is cultivated also in Australia (Johnstone, 2014) and in Europe, research being done mainly in Romania, Itally and Macedonia (Cossio et Bassi, 2011, 2013; Markovski et Velkoska-Markovska, 2015; Stănică, 2002). Jujube leaves proved to have an insecticide action against one of the major pest in the world, *Helicoverpa armigera*, by inhibiting the digestive and mitochondrial enzymes which lead to growth retarding of the larvae (Varghese et Patil, 2005) and also against *Tribolium confusum* (Vasudha, 2012).

Around the world, except its native environment, the Chinese date seems to be very resistant to pests and no major damage had been registered until now outside the Asian continent borders (Yao, 2013).

In 2013, Balikai et al. mention almost 130 pest species recorded on *Ziziphus* crop in India and specify that 177 species of insect and non-insect jujube pests were recorded around the world. Only a few of them cause substantial economic damage (Balikai et al., 2013). In

India, in an IPM governmental meeting in 2015, 10 pests are cited as pests of national significance: the fruit flies *Carpomyia vesuviana* Costa, *B. zonata* Saunders, *B. dorsalis* (Diptera: *Tephritidae*), the fruit borers *Meridarchis scyrodes* Meyr (Lepidoptera: *Carposinidae*), the green slug caterpillars *Thosea sp*. (Lepidoptera: *Limacodidae*), the grey hairy caterpillars *Thiacidas postica* Walker (Lepidoptera: *Noctuidae*), the mites *Larvacarus transitans* Ewing (Tetranychoidea: *Tenuipalpidae*), the ber beetles *Adoretus pallens* Blanchard (Coleoptera: *Scarabaeoidea*), the grape mealybugs *Maconellicoccus hirsutus* (Green) (Hemiptera: *Pseudococcidae*), the ber mealybugs *Perissopneumon tamarindus* (Green) (Hemiptera: *Pseudococcidae*), the thrips *Scirtothrips dorsalis* Hood (Thysanoptera: *Thripidae*) and the termites *Odontotermes obesus* (Isoptera: *Termatidae*) (Satyagopal, 2015). Four of these ten categories were also cited by Azam-Ali et al. in 2006, as major pests for Asia: the fruitfly, the fruit borers, the ber beetles, the mites. The same authours included in their list the bark eating caterpillars *Indarbela quadrinotata*, *I. watsoni* and *I. tetraonis* (Coleoptera: *Cerambycidae*), the hairy caterpillars *Dasychira mendosa*, *Euproctis fraterna* (Lepidoptera: *Lamantriidae*), *Thiacidas postica* (Lepidoptera: *Noctuidae*) and the lac insect *Kerria lacca* and *K. sindica* (Hemiptera: *Keridae*). Acording to Balikai, 2013, in Europe, the following jujube pests were mentioned: *Carpomyia vesuviana* Costa (by Tominic, in 1954, in Yugoslavia), *Carpomyia incompleta* (Becker) (by Monastero, in 1970, in Italy), *Bactrocera zonata* (Saunders), (by Anonimus, in 2010, in the EPPO member countries, mentioned by Sarwar, 2006 as jujube pest), *Ceratitis capitata* (Wiedemann) (by Martinez et al., in 2006, in Amposta, Spain), *Hispa sp*. (by Jolivet, in 1989, in Turkey), *Grammadera clara* Brunner von Wattenwyl, (by Liebermann, in 1970, in Spain).

In Romania, Stănică, 1997, mentions that the most important pests of *Ziziphus jujube* are *Carpomya vesuviana* (Costa) and *C. incompleta* (Beck), dipterous that lay their eggs in July under the fruit epiderma and *Carposina sasakii* (Mats.), one Lepidopterous which in China destroys 15-20% of fruits, other minor pests being *Ceratitis capitata*, *Cydia molesta* and *Polycrosis botrana*.

No major research programs have been started until now regarding the resistance to pests, except some mentioned by Azam-Ali et al., 2006, against the fruitfly *Carpomyia vesuviana* and fruit borer *Meridarchis scyrodes*.

During the last 20 years, since we first introduced the jujube tree in the experimental fields of University of Agronomic Science and Veterinary Medicine Bucharest, no pests were observed in the field, except *Ceratitis capitata* fly, starting with 2013 and no chemical applications were needed. The year 2016 was totally exceptional from the point of view of climatic conditions and pests evolution, especially for the new invasive species. Four species were damaging the crop, namely *Metcalfa pruinosa* Say, *Ceratitis capitata* (Wiedemann), *Nezara viridula* (L.) and *Halyomorpha halys* (Stal), while another 2 species producing damages were not yet identified. Using online databases, we estimate the possible risk raised by other recorded pests of jujube crop in Romania.

MATERIALS AND METHODS

The present article includes a comprehensive literature review and also presents our own research results. The literature review is based on the online and offline bibliographical references that we found on the WEB and in the University library. We used the following international databases: Web of Science - Core Collection (Journal Citation Reports, Derwent Innovations Index, Thomson Reuters), SpringerLink Journals (Springer), Scopus (Elsevier), ScienceDirect Freedom Collection (Elsevier), PROQUEST Central, Oxford Journals, CAB Abstracts, Google Scholar, Agris Fao, simple google research.

Our observations were made in the experimental field of USAMV Bucharest, in the new fruit species testing field of the Faculty of Horticulture. Regular monitoring visits were done weekly, starting from August 2016. For the presence of *Ceratitis capitata* adults, Tephri traps were used by the researchers of Research and Development Institute for Plant Protection, Bucharest, Romania and the results will be presented by them (Chireceanu et al., 2013). In laboratory, during the chinese dates morphometric determinations, we identified *C. capitata* larvae and apreciate the number of affected fruits, without precise counting. This prelimary observation will be follow-up in 2017.

To check the scientifically accepted name, spelling, alternative names and geographical distribution of pest species, we used the online Catalog of life databases, which holds essential information on the names, relationships and distributions of over 1.6 million species and the number is continuously rising. To verify the presence of a specific pest species or genus in Romania, we used the ARTHropod Ecology, Molecular Identification and Systematics database, belonging to INRA, France.

For the new non-native species *Halyomorpha halys*, as we consider it a serious threat, our laboratory launch its first citizen science action in July 2016. Warning leaflets were given directly or sent by mail to over 4000 citizens, including agricultural producers and also shared on social networks, as LinkedIn and Facebook.

RESULTS AND DISCUSSIONS

The pests of jujube crop could be grouped into four main categories, as illustrated in fig. 1.

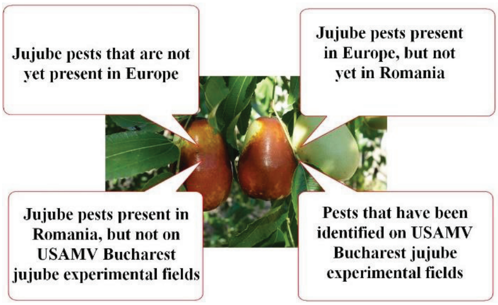


Figure 1. The four possible pests groups associated to *Ziziphus jujuba*

We present the four groups of pests in the order of their threat on jujube crop in Romania.

I. Pests that have been identified on USAMV Bucharest jujube experimental fields

The mediterranean fruit fly (MFF) - *Ceratitis capitata (Wiedemann) (*Diptera, *Tephritidae),* EPPO code CERTCA, A2 no. 105.

Ceratitis capitata is a highly invasive species, worldwide established, extremely polyphagous, having a high economic impact, affecting production, rising the pests control costs and limiting the international trade access (EPPO, 1997). According to EPPO, 2011, *C. capitata* is the most serious pest of some fruits, including citrus, especially in the countries with a warm, Mediterranean, tropical or subtropical climate, but the species is spreading to the north and the recent climate changes makes a larger area suitable for its establishment. Its presence, even as temporary population, leads to severe constraints regarding the export of fruits to uninfested areas and make *C. capitata* one of the most significant quarantine pests for the EPPO region. It originates in tropical Africa, from where it has spread to the Mediterranean area and the world. The larvae develop in fruits, in a very wide range of host plants, in the EPPO region the main hosts belonging to a large number of tree fruit crops, as apples, peaches, plums, cherry, avocados, *Citrus sp*., figs, kiwifruits, mangoes, pears etc. It has also been recorded on wild hosts. *Ceratitis capitata* is an EPPO A2 quarantine pest (EPPO, 1981), at no. 105 (EPPO, 2015) and is also of quarantine significance throughout the world. The spread to Europe, North and Eastern Africa, the Middle East, Australia and the Americas is the result of accidental transportation during the trade (CABI, 2016a).

The presence of the Mediterranean fruit fly in Romania was first time mentioned by Stanciu, 2007, on *Diospyros kaki* and described by Chireceanu et al. in 2013, following their research on 17 locations in Romania, on apricot, jujube and wild blackberry. The authors found *C. capitata* adults in five locations, on jujube in orchards and in private garden (Bucharest and Moara Domneasca, Ilfov County), on apricot in private garden (Agigea-Constanta County) and on wild blackberry bushes (Bucharest).

Regarding the attack on jujube, Chireceanu et al., 2013, states that in Bucharest the percentage of affected jujube fruits was under 10% and that the adults were collected from September to October, the first individuals being captured just when the jujube fruits start ripening.

During our observations, in 2016, in the laboratory, while weighting the jujube fruits which were previously held in plastic bags, we identified the *C. capitata* larvae, which flex and 'jump' repeatedly up to 2 cm when we pick the fruits from the bag. This behavior was previously described when the surrounding air temperature was warm and the larvae fully grown (EPPO, 2011). The degree of affected fruits was appreciated under 5%.

The morphological identification with a binocular microscope is the recommended diagnostic method and the description of the fly is provided by EPPO Diagnostics, 2011.

Biology

The eggs of *Ceratitis capitata* are laid below the skin of the fruit and hatch within 2-4 days in warm days and 16-18 days in cool weather and the larvae feed for others 6-11 days. The pupa forms in the soil, beneath the host plant and adults emerge after 6-11 warm days or longer in cooler days and adults live for up to 2 months (Christenson et Foote, 1960).

The adult flight and the transport of infested fruits are the major means of movement and dispersal to previously uninfested areas. Scientists suggest that *C. capitata* can fly at least 20 km (EPPO, 1990). Some fruits are only infested when ripe but precautions should be taken and the production should be carefully checked before transportation in another region.

Damage

The *Ceratitis capitata* larvae consume the heart of the fruit, making them inappropriate for consumption or processing. The attacked fruits have puncture marks made by the female's ovipositor and they often fall down before maturation.The fly can act also as a potential vector for *Erwinia amylovora* (Ordax et al., 2015), for other fungi, as *Rhizopus stolonifer* (Cayol et al., 1994), while Sela et al., in 2005 demonstrate that the fly is a potential vector of human pathogens as coliforms and *E. coli*.

Control

When detected, it is important to gather all fallen and infected host fruits and destroy them. The detection of *Ceratitis capitata* is done during monitoring with traps with trimedlure *(t-butyl-4(or5)-chloro-2-methyl-cyclohexane-carboxylate)* and terpinyl acetate, which attracts the males.

Malathion was long time used for chemical spraying and bait sprays (both male and female of *C. capitata* are attracted to a protein source which emanates ammonia). Bait sprays have minimal impact on natural enemies, as only the pests are attracted and killed by the chemical insecticide. Qazzaz et al. in 2015, demonstrate that extracts of entomopathogenic *Beauveria bassiana* significantly reduced the peach infestation.

Regarding the phytosanitary measures, the fruits imported from countries where *Ceratitis capitata* occurs, need to be carefully inspected and those suspected for symptoms of infestation should be cut and controlled for larvae. EPPO recommends treatments of the fruits belonging to the genus *Citrus* and *Prunus* by either cold treatment while in transit either by vapour heat, forced hot air or hot water treatment (EPPO, 1997). Fumigants as ethylene dibromide or methyl bromide are no longer recommended either because of the carcinogen effect, either because of the damaging effect on fruits or fruit shelf-life. Irradiation, a combination of methyl bromide fumigation and cold treatment and wrapping fruits in shrink-wrap film were investigated as possible methods of disinfesting procedures (Ohta et al., 1989, Jang, 1990).

The brown marmorated stink bug (BMSB) - *Halyomorpha halys (Stal) (*Heteroptera*:* *Pentatomidae)*. EPPO code HALYHA, deleted from the Alert list in 2013

Halyomorpha halys is a highly polyphagous pest, attacking mainly fruit trees and vegetables, but also field crops, some ornamentals and weeds. BMSB has become a major nuisance pest in the USA, in mid-Atlantic region and Pacific Northwest, due to its overwintering behavior which leads to unimaginable homes invasions (Inkley, 2012). In the mid-Atlantic region, serious crop losses have been reported for apples, peaches, sweet corn, peppers, tomatoes and row crops such as field maize and soybeans since 2010. Paula et al., 2016 consider this pest a devastating invasive species in the USA, as the direct losses produced only in the mid-Atlantic apples orchards alone, in the year 2010, were about $37 million. In Europe, the damages produced by *H. halys* in Italy, in 2016, were approximated around 1 billion Euro (Fontana, 2016). For humans, allergic reactions and dermatitis have been mentioned (Anderson, 2012).

In Europe, the first specimen was captured in a light trap in 2004 in Liechtenstein at Balzers (EPPO, 2008), afterwards in 2007 in Switzerland (Wermelinger et al., 2008) and its evolution was silent until 2015, when it became invasive and major damages were reported, as in Italy, where despite all control measures applied, pest feeding caused severe damage to fruit crops, especially pear (EPPO, 2016). The pest was mentioned for the first time in 2011 in Germany (Heckmann, 2012) and Greece (Milonas et Partsinevelos, 2014) in 2012 in Italy (EPPO, 2013a) and France (EPPO, 2013b), in 2013 in Hungary (Vétek, 2014), in 2014 in Russia (EPPO 2016a), in 2015 in Austria (EPPO, 2016b) and Serbia (EPPO, 2016c).

In Romania, Macavei et al., 2015 first time described this species, found in Bucharest in September 2014, but they state that its presence could date back to at least 1-2 years ago, due to the fact that individuals were found at several kilometers away. Our observations confirm this theory and leads to the hypothesis that the cohabitation habit between *Nezara viridula* and *Halyomorpha halys* species is the misleading factor in the late identification of *H. halys* specimens in Romania. In 2016, in the USAMV Bucharest corn testing fields, goji testing fields and edible roses testing fields, the two species were always observed in cohabitation and they produced crop losses of 100% to goji crop, starting with the month of august (Ciceoi et al., 2016a,b). On the corn field, the estimated *H. halys* density/m^2 plant was 25.5 individuals on the field border area and 9.75 individuals in the field interior area, which indicates an exponential growth of the marmorated stink bug populations. On jujube experimental field, the adults of *H. halys* were observed since early September and we consider that the main two reasons for this late occurrence are the fact that the goji crop found in the vicinity was more “feeding” attractive and the population was fix until the complete fruit depreciation of the fruits and the fact that the fruits of jujube start to ripen in the first decade of September.

Biology

The biology of BMSB depends on its environment; it has up to five generations/year in southern China, one or two generations in USA and one generation had been reported until now in Europe (CABI, 2016c). Although the overwintering adults become active in April, due to the high demand of temperature, the oviposition peak in Europe starts at beginning of July (Haye et al., 2014) and then continue until the end of September. The eggs are laid in clusters of around 30 eggs (CABI, 2016c), on the inner leaves surfaces and a female may lay in average 79 eggs, maximum observed being 160 eggs (Haye et al., 2014). The species has 5 larval instars and the adults emerge in 60 to 131 days, depending on the temperature fluctuations. In controlled conditions, at 30°C, the cycle from egg to adult only lasted only 33.2 days and below 15 and above 35°C no development was observed (Nielsen et al., 2008, Haye et al., 2014). More than 300 host plants have been recorded, the most severely damaged being the tree fruits and small fruits, the vegetables, especially tomatoes, ornamentals and field crops (Nielsen et al., 2008).

Damage

The attacked fruits present feeding punctures, caused by the plant juices sucking, which lead to suberifications, the formation of necrotic areas and sometimes to deliquescent fruit pulp (Rice et al., 2014). The adults feed on fruits and the nymphs feed on leaves, stems and fruit. In 2016, in Bucharest some hobby tomatoes crops were completely destroyed by the BMSB, while at the USAMV Bucharest experimental field heavy infestations were observed on corn and goji crops, the goji fruits being severely distorted and quickly become blackish and juiceless, unmarketable. In Asia, *H. halys* cause significant damage to soybean, in Northern Japan to apple, in Italy to pear. In addition to plant damage, BMSB is a nuisance to humans because the overwintering adults aggregate in buildings and houses. *H. halys* has a strong flight capacity, including for long distances (more than 5 km/day), particularly in the summer generation, so the spreading capacity is very high.

Control

Monitoring and surveillance of the pest spreading in new areas are capital and should be followed by immediate eradication measures. The IPM programs for this pest are correlated with the number of generations per year and the peak of the last adult population, which is the most damaging stage (Nielsen et al., 2008). The black light traps and baited black pyramid traps (with methyl (2E,4E,6Z)-decatrienoate) have been successfully used to monitor *H. halys* (Leskey et al., 2012). The H. *halys* aggregation pheromone has been identified by Zhang et al., 2013. Leskey et al., 2012, tested the efficacy of 37 insecticide treatments and proved that dimethoate, malathion, bifenthrin, acephate, permethrin, methidathion, endosulfan, methomyl, chlorpyrifos and fenpropathrin have a greater potential for controlling BMSB.

Biological control methods are desirable, but as *H. halys* is a newly established invasive pest, the lack of knowledge combined with the lack of predators and parasites in the newly invaded areas makes the chemical control methods preferable. Some observations mention *Harmonia axyridis* and earwigs being efficient in eggs predation.

Citrus flatid plant-hopper - *Metcalfa pruinosa Say (*Homoptera*: Flatidae),* EPPO code: METFPR, no list

The citrus flatid plant-hopper is an extremely polyphagous pest, more than 300 hosts being mentioned until the present moment (Vlad et Grozea, 2016). It is a major nuisance pest in parks and gardens and produce qualitative depreciation of agricultural products, caused by the feeding with the sap of plants and by the massive productions of waxy secretions and honeydew, when in numerous populations (CABI, 2016d). The species originate from eastern North America and was first time mentioned in Italy in 1979 (Bărbuceanu, 2015, Strauss, 2010) and is now present in at least 15 European counties (Strauss, 2010, EPPO, 2016). Recently was discovered also in the eastern paleartic area, in Korea, where it imedialtely became a quarantine pest (Kim et al. 2011)

In Romania *M. pruinosa* was first time mentioned by Preda et Skolka, 2009, in the city of Constanta, in 2009, then in 2010 in Timisoara (Gogan et al. 2010, Grozea et al., 2011) and in 2011 in Bucharest, (Chireceanu et Gutue, 2011).

Our observation in the jujube experimental field showed low infestations and only on the lower leaves of the trees, having no influence on the fruit quality. Still, considering its evolution on other crops, we recomend a throughout monitoring of the *M. pruinosa* activity all year long.

Biology

According to Mead, 2014, *Metcalfa pruinosa* overwinters as eggs, hatching starting early in the spring. First adults emerge in around 70 days after the hatching and the species has only one generation per year. The species has five larval instars. In Romania, the larvae occur in the second half of May and the first adults can be seen in late July. An overlap of the stages usually happen (Bărbuceanu, 2015).

Damage

Although new and non-native pest for Europe, considering the low economic impact of the species in its area, *M. pruinosa* was not considered quarantine pest by EPPO. Although Mead, 2014 considers the pest does very little damage to plants, in Europe, Strauss, 2010 mentions serious qualitative damage of grapes and quantitative damage in soybean in Italy. For viticulture and fruit growing, the negative impact on quality is due to the *M. pruinosa*'s honeydew secretions, wich stimulate the black sooty mold development Strauss, 2010.

Control

Limiting the spreading of this pest is difficult, due to its very high mobility, very large host plants spectrum and waxy protection of the individuals. Bărbuceanu, 2015, conclude that the insecticide with the highest effectiveness is the mixture of imidacloprid 75 g/l, deltamethrin 10 g/l, lambda cyhalothrin 50 g/l, followed by a treatment with pirimiphos methyl 500 g/l, at 3-5 days after the first one. Still, the chemical treatments should be avoided, as the broad spectrum insecticides kills the natural beneficial species. The biological control with *Neodrynus typhlocybae* is assumed to be advantageous by Strauss, 2012.

Southern Green Stink Bug - *Nezara viridula* *(L.) (Heteroptera: Pentatomidae)* *EPPO code NEZAVI*

The southern green stink bug originates from Ethiopia and East Africa, although some authors believe it came from southern Asia (Grozea et al, 2012). It is spread now in the tropical and subtropical regions of Europe, Asia, Africa and the Americas (Squitier, 2013). The first reports of pest in Europe date from 1998, in Italy (CABI, 2016b). It is a highly polyphagous species, causing economic damage to legumes, soybean and beans (Portilla et al., 2015) but also fruits and vegetables.

In Romania, qualitative and quantitative depreciations of tomatoes were described by Grozea et al. in 2012, in Timisoara area while in Muntenia area the presence of the pest is stated in 2015 (Kurzeluk et al. 2015). The author mentions that in the Bucharest area *Nezara viridula* was identified on a goji experimental field since 2011.

The adults of *N. viridula* were observed on jujube experimental field since the beginning of September. The biology and ecology of *N. viridula* seem to be closely related to the *H. halys*'s one, as these two species cohabits. The vicinity of the depleted goji trial field and the jujube fruit maturity are considered the two main reasons for the late occurrence of the adults in the testing field. No major damages were noticed following the feeding on fruits, but further research is recommended, as the biology of the pest might change in the long and warm autumns, when the fruit start to ripe.

Biology

In the warm climates, the southern green stink bug may have four generations per year while in Europe just one generation had been mentioned. Overwinters as an adult under the bark of trees or leaf litter. In spring, after the feeding, the oviposition may start. In US, eggs have been found starting the second week of April till December. One female of *Nezara viridula* could maximum lay 260 eggs (Squitier, 2013). Its development includes five larval instars.

Damage

The damages include drop and malformation of the fruits (Panizzi, 2008) on the rostrum inserting points into the fruit or growing shoots tissues. The damages are even higher due to the digestive enzymes introduced while feeding (Grozea et al., 2015). Black spots or suberification may be observed on the mature fruits, while the younger fruits usually drop out of the plant.

Control

Although not considered a major pest, the economic thresholds for different crops were determined in the USA. For example, in soybeans, 36 stink bugs per 100 swings of a net, for cowpea 5000 southern green stink bugs per ha and 3 - 4 stink bugs per 100 swings for cotton are considered the upper limits before chemical interventions. Trap crops are considered having great potential. As biological control means, two parasites were introduced, a tachinid fly, *Trichopoda pennipes*, which parasitizes the adults and the wasp *Trissolcus basalis*, which parasitizes the eggs.

II. Jujube pests that are present in Romania, but have not been identified on USAMV Bucharest jujube experimental fields

Among the pests that have been mentioned in the literature as attacking jujube crop and that exist in Romania, but we did not identify it on the jujube crop yet, two are more important:

a. the leopard moth, *Zeuzera pyrina*, (Lepidoptera*, Cossidae*)*,* EPPO code ZEUZPY, which is included in EPPO Alert list since 2001 and

b. the oriental fruit moth, *Grapholita molesta*, (Lepidoptera*, Tortricidae*)*,* EPPO code LASPMO, formerly on A1/A2 list.

III. Jujube pests that are present in Europe, but not yet in Romania

Among the pests that have been mentioned in the literature as attacking jujube crop and that exist in Europe, but not yet in Romania, the ber fruit flies *Carpomya vesuviana* and *Carpomya incompleta (Diptera, Tephritidae)* EPPO code CARYVE, formerly on A1/A2, are the most dangerous. *C. vesuviana* was found in Bosnia-Herzegovina, Italy and Turkey, while *C. incompleta* was mentioned in South of Europe, Israel, France and Italy. (Vadivelu, 2014, Pollini, 2014, fera, 2016, catalogue of life.org, 2016)

The ber fruit fly, *Carpomya vesuviana* Costa, 1854 (Diptera: Tephritidae) is the most destructive pest of *Ziziphus* crop in its area of distribution, that includes Bangladesh, China, Georgia, India, Indian Ocean Islands, Iran, Mauritius, Oman, Pakistan, Southern Europe, Turkmenistan, Turkey, and Uzbekistan (Amini et al., 2014, Vadivelu 2014).

The bug *Apolygus lucorum* (Meyer-Dur, 1843) (*Miridae: Hemiptera*), is another major pest of jujube crop in Asia. Overwintering *A. lucorum* eggs are primarily laid in the summer pruning wounds of Chinese date trees. It was reported in Europe in Britain, Denmark, Germany, Italy, Macedonia, Poland, Russia, Spain, Sweden, Switzerland, and Turkey, (catalogoflife.org, 2016, Pan et al., 2014). Another jujube pest is *Lygus pratensis* L. (*Miridae: Hemiptera*), which, according to the catalogue of life, was mentioned in Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Georgia, Germany, Greece, Iran, Italy, Hungary; Britain; Corsica, Macedonia, Siberia, Sweden and Turkey.

IV. Jujube pests that are not yet present in Europe

The most dangerous pest for jujube crop, but also for the entire fruit production in Europe is the Peach fruit moth - *Carposina sasakii (*Lepidoptera*: Carposinidae),* EPPO code CARSNI, A1 list: No. 163 (CABI, 2016e)

Also known as *Carposina niponensis,* the peach fruit moth can be easily mistaken as *Cydia pomonella* or *Cydia molesta*. Considered as one of the most important pests of pome fruits in the Far East, the quarantine pest can damage pears up to 100% in some cases, while apples are less heavily infested, in a degree of 40-100% (EPPO QP, 1988). Special phytosanitary measures are imposed for all *Chaenomeles*, *Crataegus*, *Cydonia*, *Eriobotrya*, *Malus*, *Prunus*, *Pyrus* and *Ziziphus* plants with roots imported from Asian country where this pest occurs. Additionally, the consignment should have been kept under conditions which prevent a reinfestation by the organism (EPPO, 1990). The growth of the larvae in the apple fruits was carefully examined by Koizumi, 2010.

CONCLUSIONS

Until the present moment, in Romania, *Ziziphus jujube* keeps its status of crop without major phytosanitary problems. All four identified pests produced a low to inexistent degree of damage.

Major attention should be given to the plants intended for planting imported from Asian countries. Although it might seem a good opportunity, importing plants with a questionable origin or doubtful phytosanitary certificate might have a severe economic impact on the fruit-growing areas of the EPPO region.

Considering that many alien invasive polyphagous pests start producing severe damages in Europe and USA and the recent climate changes, we recommend a thorough monitoring program and a detailed pest biology observation program.

REFERENCES

Amini A. Sadeghi H. Lotfalizadeh H. Notton D., 2014. Parasitoids Hymenoptera: Pteromalidae, Diapriidae) of *Carpomyia vesuviana* Costa (Diptera: Tephritidae) in South Khorasan province of Iran. Biharean Biologist 8(2):122-123.

Anderson B.E., Miller J.J., Adams D.R., 2012. Irritant contact dermatitis to the brown marmorated stink bug, *Halyomorpha Halys*. Dermatitis, 23(4):170-172.

Azam-Ali S., Bonkoungou E., Bowe C., deKock C., Godara A., Williams J.T., 2006. Ber and other jujubes. International Centre for Underutilised Crops, Southampton, UK.

ARTHropod Ecology, Molecular Identification and Systematics *database,* accessed on 09.10.2016.

Balikai R.A., Kotikal Y.K., Prasanna P.M., 2013. Global scenario of insect and non-insect pests of jujube and their management Options. Proc. 2nd International Jujube Symposium Acta Hort. 993:253 - 277.

Bărbuceanu D., Mihăescu C. F., 2015. New data about the control of the planthopper *Metcalfa pruinosa* (Say 1830) (Hemiptera: Flatidae) in the town of Piteşti (Argeş County). Annals of the University of Craiova - Agriculture, Montanology, Cadastre. XLV:26-31.

CABI, 2016a. *Ceratitits capitata* (Mediterranean fruit fly), http://www.cabi.org/isc/datasheet/12367

CABI, 2016b. *Nezara viridula* (green stink bug) http://www.cabi.org/isc/datasheet/36282#20163102287.

CABI, 2016c. *Halyomorpha halys* (Marmorated Stink bug), http://www.cabi.org/isc/datasheet/27377.

CABI, 2016d. *Metcalfa pruinosa* (frosted moth-bug), http://www.cabi.org/isc/datasheet/35054.

CABI, 2016e. *Carposina sasakii* (peach fruit moth), http://www.cabi.org/isc/datasheet/11401

Cayol J.P.; Causse R.; Louis C.; Barthes J., 1994. Medfly *Ceratitis capitata* as a rot vector in laboratory conditions. Journal of AppEnt 117, 338-343.

Ciceoi R., Mardare E. Ș., Teodorescu E., Dobrin I., 2016a. The status of brown marmorated stink bug,

Halyomorpha halys, in Bucharest area. JHFB, 20(4):18-25, DOI: 10.5281/zenodo.267978
Ciceoi R, Mardare E.S., 2016b The risks assessment of *Aceria kuko* (Kishida) and *Halyomorpha halys* (Stal) pests for the Romanian goji growers. Poster. DOI: 10.5281/zenodo.345966
Chireceanu C., Gutue C., 2011. *Metcalfa pruinosa (Say) (Hemiptera: Flatidae)* identified in a new south eastern area of Romania (Bucharest area), Romanian Journal of Plant Protection, 4:28-34.
Chireceanu C., Stănică F., Chiriloaie A. 2013. The Presence of the Mediterranean fruit fly *Ceratitis Capitata* (Wied.), (Diptera: Tephritidae) In Romania, Romanian Journal of Plant Protection. VI:92-97.
Christenson L.D., Foote R.H., 1960. Biology of fruit flies, Annual Review of Entomology 5:171-192.
Cossio F., Bassi G., 2011. Prospettive di diffusione in Italia di varietà cinesi di giuggiolo a frutto grosso, Frutticoltura. 11:58-61.
Cossio F., Bassi G., 2013. Field Performance of Six Chinese Jujube Cultivars Introduced and Tested In Northern Italy. Acta Hortic. 993:21-28.
EPPO, 1981. Data sheets on quarantine organisms No. 105, *Ceratitis capitata*. Bulletin EPPO Bulletin 11 (1).
EPPO, 1990. Specific quarantine requirements. EPPO Technical Documents No. 1008.
EPPO⁄CABI, 1997. *Ceratitis capitata*. Quarantine Pests for Europe, Wallingford (GB), 2nd edn, 146–152.
EPPO, 2011. Diagnostics. Ceratitis capitata PM 7/104(1). OEPP/EPPO Bulletin 41:340–346.
EPPO Services, EPPO A1 And A2 Lists Of Pests Recommended For Regulation As Quarantine Pests, 2015. EPPO.
EPPO, 2013a. First report of *Halyomorpha halys* in Italy (2013/108), EPPO Reporting Service, 05:10-11.
EPPO, 2013b. *Halyomorpha halys* continues to spread in the EPPO region: first reports in France and Germany (2013/109), EPPO Reporting Service, 05:11.
EPPO, 2016a. First report of *Halyomorpha halys* in Russia (2016/148), EPPO Reporting Service, 08:12.
EPPO, 2016b. First report of *Halyomorpha halys* in Austria (2016/150), EPPO Reporting Service, 08:13.
EPPO, 2016c. First report of *Halyomorpha halys* in Serbia (2016/151), EPPO Reporting Service, 08:14.
Fontana L., 2016. Question for written answer to the Commission. Subject: Damage to Italian agriculture: the case of the brown marmorated stink bug, Parliamentary questions.
Gogan A., Grozea I., Vîrteiu A. M., 2010. *Metcalfa pruinosa* Say (Insecta:Homoptera: Flatidae) –first occurrence in western part of Romania. Res. J. Agric. Sci., 42(4):63-67.
Golmohammadi F., 2013. Medicinal plant of Jujube (*Ziziphus jujuba*) and its indigenous knowledge and economic importance in desert regions in east of Iran: situation and problems, Tech J Engin & App Sci., 3 (6): 493-505.
Grozea I., Gogan A., Virteiu A.M., Grozea A., Stef R., Molnar L., Carabet L., Dinnesen S., 2011. *Metcalfa pruinosa* Say (Insecta: Homoptera: Flatidae): A new pest in Romania, African Journal of Agricultural Research Vol. 6(27), 5870-5877.
Grozea I., Ştef R., Virteiu A.M., Cărăbeţ A., Molnar L., 2012. Southern green stink bugs (*Nezara viridula* L.) a new pest of tomato crops in western Romania. R. Journal of Agricultural Science, 44(2):24-27.
Grozea I., Virteiu A.M., Ştef R., Cărăbeţ A., Molnar L., Florian T., Vlad M., 2015 Trophic Evolution of Southern Green Stink Bugs (*Nezara viridula l.*) in Western Part of Romania, Bulletin UASVM Horticulture 72(2), 371-375.
Gupta A.K., 2004. Origin of agriculture and domestication of plants and animals linked to early Holocene climate amelioration. Current Science. 87:54- 59.
Haye T., Abdallah S., Gariepy T., Wyniger D., 2014, Phenology, life table analysis and temperature requirements of the invasive brown marmorated stink bug, *Halyomorpha halys*, in Europe. J. Pest Sci. 87:407–418.
Heckmann R., 2012. Erster Nachweis von *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) für Deutschland. Heteropteron 36:17-18.
Inkley DB, 2012. Characteristics of home invasion by the brown marmorated stink bug (Hemiptera: Pentatomidae). J. Entomol. Sci. 47:125-130.
Jang, E.B., 1990. Fruit fly disinfestation of tropical fruits using semipermeable shrink-wrap film. Acta Horticulturae No. 269, abstract.
Johnstone R., 2014, Development of the Chinese jujube industry in Australia, Rural Industries Research and Development Corporation, Publication No. 14/001.
Kim Y., Kim M., Hong K-J., Lee S., 2011. Outbreak of an exotic flatid, *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae), in the capital region of Korea. Journal of Asia-Pacific Entomology, 14:473–478.
Koizumi M, Ihara F, Yaginuma K, Kano H, Haishi T., 2010. Observation of the peach fruit moth, *Carposina sasakii*, larvae in young apple fruit by dedicated micro-magnetic resonance imaging. Journal of Insect Science 10:145.
Kurzeluk D.K., Fătu Ac., Dinu Mm, 2015. Confirmation of the presence of the Southern Green Stink Bug, *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Pentatomidae), in Romania, Romanian Journal for Plant Protection, Vol. VIII.
Leskey T.C., Hamilton G.C., Nielsen A.L., Polk D.F., Rodriguez-Saona C., Bergh J.C., Herbert D.A., Kuhar T.P., Pfeiffer D., Dively G.P., Hooks C.R.R., Raupp M.J., Shrewsbury P.M., Krawczyk G., Shearer P.W., Whalen J., Koplinka-Loehr C., Myers E., Inkley D., Hoelmer K.A., Lee D.-H., Wright S.E., 2012, Pest Status of the Brown Marmorated Stink Bug, *Halyomorpha Halys* in the USA. Outlooks Pest Manag. 23:218–226
Li J.W., Fan L. P., Ding S. D., Ding X. L., 2007. Nutritional composition of five cultivars of chinese jujube. Food Chemistry 103:454-460.
Liu M.J., Zhao Z.H., 2009, Germplasm resources and production of Jujube in China, Proc 1st Int`l jujube Symp, Acta Hort 840:25-31.
Liu M.J., Zhao J., Cai Q.L., Liu G.C., Wang J.R., Zhao Z.H., Liu P, Dai L., Yan G, Wang W.J., Li X.S., Chen Y., Sun Y.D., Liu Z.G., Lin M.J., Xiao J., Chen Y.Y., Li X.F., Wu B., Ma Y., Jian J.B., Yang W.,

Yuan Z., Sun X.C., Wei Y.L., Yu L.L., Zhang C., Liao S.G., He R.J., Guang X.M., Wang Z., Zhang Y.Y., Luo L.H., 2014. The complex jujube genome provides insights into fruit tree biology**.** Nature Comm., 5: 5315

Macavei L.I., Bâeţan R, Oltean I, Florian T, Varga M, Costi E, Maistrello L., 2015. First detection of *Halyomorpha halys* Stål, a new invasive species with a high potential of damage on agricultural crops in Romania. Lucrări Ştiinţifice seria Agronomie 58(1):105-108.

Markovski A., Velkoska-Markovska L., 2015. Investigation of the morphometric characteristics of Jujube types (*Zizyphus jujuba* Mill.) fruits in Republic of Macedonia. Genetika, Vol. 47(1):33-43.

Mead F.W., 2014. Citrus Flatid Planthopper*, Metcalfa pruinosa (Say) (Insecta: Hemiptera: Flatidae)*, Florida Department of Agriculture and Consumer Service-Division of Plant Industry: Gainesville FL.

Milonas P.G., Partsinevelos G.K. 2014. First report of brown marmorated stink bug *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) in Greece. Bulletin OEPP/EPPO Bulletin 44(2):183-186.

Nielsen A.L., Hamilton G.C., Matadha D., 2008, Developmental Rate Estimation and Life Table Analysis for Halyomorpha halys (Hemiptera: Pentatomidae). Environ. Entomol. 37:348–355.

Ohta A.T.; Kaneshiro K.Y.; Kurihara J.S.; Kanegawa K.M.; Nagamine L.R., 1989. Gamma radiation and cold treatments for the disinfestation of the Mediterranean fruit fly in California-grown oranges and lemons. Pacific Science 43:17-26.

Ordax M., Piquer-Salcedo J.E., Santander R.D., Sabater-Muñoz B., Biosca E.G., López M.M., 2015. Medfly *Ceratitis capitata* as Potential Vector for Fire Blight Pathogen *Erwinia amylovora*: Survival and Transmission. PLoS ONE 10(5):e0127560.

Pan H, Liu B, Lu Y, Desneux N., 2014. Identification of the key weather factors affecting overwintering success of *Apolygus lucorum* eggs in dead host tree branches. PLoS ONE 9(4): e94190.

Panizzi A.R., 2008. Southern green stink bug*, Nezara viridula* (L.) (Hemiptera: Heteroptera: Pentatomidae). Encyclopedia of Entomology pp. 3471.

Paula D.P., Togawa R.C., Costa M.M.C., Grynberg P., Martins N.F., Andow D.A., 2016. Identification and expression profile of odorant-binding proteins in *Halyomorpha halys* (Hemiptera: Pentatomidae), Insect Molecular Biology 1-15.

Preda C., Skolka M., 2009. First record of a new alien invasive species in Constanta - *Metcalfa pruinosa* (Homoptera: Fulgoroidea). In: Paltineanu C. (Ed.) Lucrarile Simpozionului Mediul si Agricultura in regiunile aride: prima editie. Estfalia, 141-146.

Preeti S., Tripathi S., 2014. A phytopharmacological review on "*Ziziphus jujuba*", Int. J. Res. Dev. Pharm. L. Sci., 3(3):959- 966.

Pollini, A., Cravedi, P., 2014 *Carpomya vesuviana* A. Costa (Diptera: Tephritidae Trypetinae Carpomyini) from jujube tree in Emilia-Romagna (Northern Italy), Redia-Giornale Di Zoologia, 97:117-118.

Portilla M., Snodgrass G., Streett D. Luttrell R., 2015. Demographic Parameters of *Nezara viridula* (Heteroptera: Pentatomidae) Reared on Two Diets Developed for *Lygus spp*., J. Insect Sci. 15(1):165.

Rice K., Bergh C., Bergman E., Biddinger D., Dieckhoff C., Dively G., Fraser H., Gariepy T., Hamilton G., Haye T., Herbert A., Hoelmer K., Hooks C., Jones A., Krawczyk G., Kuhar T., Mitchell W., Nielsen A.L., Pfeiffer D., Raupp M., Rodriguez-Saona C., Shearer P., Shrewsbury P., Venugopal D., Whalen J., Wiman N., Leskey T., Tooker J., 2014. Biology, ecology and management of brown marmorated stink bug (*Halyomorpha halys*) J. Integr. PestManage. 5(3):1-13

Qazzaz F.O., Al-Masri, M.I., Barakat, R.M., 2015. Effectiveness of *Beauveria bassiana* native isolates in the biological control of the Mediterranean fruit fly (*Ceratitis capitata*). Advances in Entomology.3:44-55

Sarwar M., 2006. Incidence of Insect Pests on Ber *(Zizyphus jujube)* Tree. Pakistan J. Zool., vol. 38(4):261-263.

Sela S., Nestel D., Pinto R., Nemny-Lavy E., Bar-Joseph M., 2005. Mediterranean fruit fly as a potential vector of Bacterial Pathogens. Applied and Environmental Microbiology, Vol. 71 (7):4052–4056.

Stanciu I., 2007. Comportarea unor soiuri de kaki în condiţiile Câmpiei Române" Ph.D thesis. Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania.

Stănică F., 1997. Curmalul chinezesc (*Ziziphus jujuba* Mill.), specie pomicolă pentru partea de sud a României (Chinese date (*Ziziphus jujuba* Mill.), fruit specie for the southern part of Romania). Hortinform.

Stănică F., 2002. Multiplication of Chinese date (*Ziziphus jujuba* Mill.) using conventional and *in vitro* techniques, XXVIth Congress ISHS, 11-17, Toronto, Canada, 303-304.

Stănică F., 2009. Characterization of Two Romanian Local Biotypes of *Ziziphus jujube,* ISHS Acta Horticulturae 840:259-262.

Strauss G., 2010. Pest risk analysis of *Metcalfa pruinosa* in Austria. Journal of Pest Science, 83:381-390.

Strauss G., 2012: Environmental risk assessment for *Neodryinus typhlocybae*, biological control agent against *Metcalfa pruinosa*, for Austria. European Journal of Environmental Sciences, 2(2):102-109.

Squitier J.M., 2013. Southern Green Stink Bug, *Nezara viridula* (Linnaeus) (Insecta: Hemiptera: Pentatomidae), UF/IFAS Extension Service, University of Florida, EENY-016 (IN142).

Varghese J., Patil M.B., 2005. Successive use of proteinase inhibitor of *Zizyphus Jujuba* leaves for effective inhibition of *Helicoverpa armigera* gut enzymes and larval growth, Biosciences Biotechnology Research Asia 3(2):367-370.

Lingampally V., Solanki V.R., Jayaram V., Kaur, A., Raja S.S., 2012. Betulinic acid: a potent insect growth regulator from *Ziziphus jujuba* against *Tribolium confusum*. Asian J. Plant Sci. Res. 2(2):198-206.

Vétek G., Papp V., Haltrich A., Rédi D., 2014. First record of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Heteroptera:

Pentatomidae), in Hungary, with description of the genitalia of both sexes. Zootaxa 3780(1):194-200.
Vadivelu K., 2014. Biology and management of ber fruit fly, *Carpomyia vesuviana* Costa (Diptera: Tephritidae): A review. African Journal of Agricultural Research 9(16):1310-1317.
Vlad M., Grozea I., 2016. Host Plant Species of the Cicada *Metcalfa Pruinosa* in Romania, Bulletin UASVM series Agriculture 73(1):131-137.
Yao, S. 2013. Past, present and future of jujubes-Chinese dates in the United States. HortScience, 48:672-680.
Wermelinger B, Wyniger D., Forster B., 2008. First records of an invasive bug in europe: *Halyomorpha halys* Stål (Heteroptera: Pentatomidae), a new pest on woody ornamentals and fruit trees? Bulletin De La Société Entomologique Suisse, 81:1–8
***species details: *apolygus lucorum* (meyer-dur, 1843), http://www.catalogueoflife.org/col/details/species/id/7fb6162f963b017e91741263c7764e20
***species details: *carpomya vesuviana* costa, 1854 http://www.catalogueoflife.org/col/details/species/id/ba931ddef181c172c517e6d6644301a5
***species details: lygus *pratensis* (linnaeus, 1758) http://www.catalogueoflife.org/col/details/species/id/1d6b9b22b095fa0df3d3c8e92e62a509
***https://gd.eppo.int/taxon/METFPR
***https://gd.eppo.int/taxon/NEZAVI
***https://gd.eppo.int/taxon/CERTCA

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDY ON THE RELATED CRACKING-RESISTANT GENES IN CHINESE JUJUBE

Yong-Xiang REN[1], Lian-Ying SHEN[1], Xiao-Ling WANG[1], Yao-WANG[1], Chun-Mei YAN[1], Li-Hui MAO[2], Yong-Min MAO[1]

[1]Agricultural University of Hebei, Lingyusi Street, No. 289, 071001, Baoding, China
[2]Renxian Agriculture Bureau, Guangming Road, NO.216, 055151, Xingtai, China

Corresponding author email: mym63@126.cn

Abstract

The problem of fruit cracking for Chinese jujube (Zizyphus jujuba Mill.) causes serious yield losses in China, however, the existing prevention and control measures are hard to solve it. Two groups of high resistant cracking type and easy cracking type for the filial generation of Dong Zao × Linyili Zao were divided in the study. Two allelic genetic pool and the related genes cDNA libraries for the cracking-resistant characters to be specifically expressed were built to conduct the preliminary feasibility study on the genes related to the resistance of fruit-cracking through the differently expressed genes. The sequencing production of the two group transcriptomes were received more than 4.5 Gb and 4.6 Gb data after removing the low quality segments, and conducted 45,401,606 and 46,468,222 times reading, the Q20 (the base ratio no less than 20) of the two libraries were both greater than 96%. After comparing the genetic expression within two groups, 391 items of differently expressed genes (DEGs) were filtered. Genome function annotation on the 391 items DEGs were conducted and the results showed that there were 92 items genes added, 45 items defined, and 299 items gene without annotation. The annotated genes probably involving in jujube cracking phenomenon were Aquaporin PIP, Tubulin, Calreticulin and Calmodulin.

Key words: *Chinese jujube, fruit cracking, cracking-resistant genes, RNA-Seq.*

INTRODUCTION

The phenomenon of fruit cracking on jujube is widespread, which causes serious yield losses, however, the existing prevention and control measures cannot fundamentally solve the problems. The researches showed that there were great differences between different varieties of jujubes on fruit cracking resistance (Mao et al., 1998；Wang et al., 2011；Yuan et al., 2013). So, it is effective means to solve the fruit cracking by breeding new varieties with high resistant cracking in Chinese jujube, the molecular marker assisted selection is a important methods in plant breeding.

Researches on the genes related to fruit cracking characteristics have been reported, such as MdExp3 gene in apples (Wakasa et al., 2003; Kasai et al., 2008), β-galactosidase gene (TBG6) in tomatoes (Moctezuma et al., 2003), Expansin gene - LcExp1gene and LcExp2 - in litchi pericarp (Wang et al., 2006), which closely related to cell wall loosening gene and fruit cracking gene. With the rapid development of high throughput sequencing, the function of transcriptome sequencing analysis has opened new avenues for the study of fruit cracking gene. Li used the high throughput sequencing technology to do the transcriptome sequencing analysis on crack resistance and easy to crack pericarps, through the analysis of the differences in gene expression, there were 67 candidate genes about fruit cracking screened out (Li et al., 2014), including 4 water transportation related genes (LcAQP, 1; LcPIP, 1; LcNIP, 1; LcSIP, 1), 5 genes related to Gibberellic Acid (Gibberellic Acid, GA) metabolism (LcKS, 2; LcGA2ox, 2; LcGID1, 1), 21 Abscisic Acid (Abscisic Acid , ABA) metabolism related genes (LcCYP707A, 2; LcGT, 9; Lcβ-Glu, 6; LcPP2C, 2; LcABI1, 1; LcABI5, 1), 13 genes related to calcium transportation (LcTPC, 1; Ca^{2+}/H^+ exchanger, 3; Ca^{2+}-ATPase, 4;

LcCDPK, 2; LcCBL, 3), and 24 cell wall metabolism related genes (LcPG, 5; LcEG, 1; LcPE, 3; LcEXP, 5; Lcβ-Gal, 9; LcXET, 1).

The main objectives of this study are (1) building two near-isogenic pools with the hybrid offspring of Dong Zao × Linyili Zao, which have the same genetic background; (2) establishing jujube resistance to cracking fruit traits specific related genes expression cDNA library, and to do the sequencing analysis generating the relevant ESTs sequences; (3) sequencing the differential expression genes, and to do gene function analysis and prediction on the ESTs sequences. This will provide references for the study of new genes and new germplasm about jujube and related plants, and it will have important significance to breed new cracking-resistance varieties through molecular techniques.

MATERIALS AND METHODS

Plant material

The supplied samples were 12 year-old hybrid offspring of Dong Zao × Linyili Zao in Jujube breeding base located in Wangcun town, Daming County, Hebei Province).

Two group of 12 cracking-resistance (cracking fruit rate < 5%, group A) and 12 sensitive-cracking (cracking fruit rate > 80%, group B) hybrid offspring were divided, according to the survey results of consecutive years cracking fruit rate on the hybrid offspring.

During young fruit stage (at the end of the 20 days after flowering), 10 young fruits of each type were picked and covered with silver paper immediately, then put into liquid nitrogen to quick-freeze. The samples were taken back to laboratory and preserved in an icebox (T=-80°C) for subsequent analyses.

RNA extraction

The fruit RNA of the offspring was extracted using the RNeasy plant mini kit (Qiagen); On-column DNase digestion with the RNase-Free DNase set (Qiagen) was performed to remove contaminated DNA.

Twelve mixed Chinese jujube cDNA samples were prepared for each group, and then the RNA samples were sent to prepare library with NEB RNA library prep kit and sequence with Illumina HiSeq2000. The RNA-Seq data were subjected to bioinformatic analysis.

Sequence bioinformatics

The raw reads were filtered with FASTQ_Quality_Filter tool from the FASTX-toolkit. Reads both with more than 35bp and having a quality score higher than 20 were kept. Then all the valid reads of A and B samples were combined to perform de novo splicing by paired-end method with Trinity software (Grabherr et al., 2011). The longest transcript per locus was used as a unigene, and as a result we got 94,984 unigenes.

Several complementary approaches were utilized to annotate the unigenes, which were conducted using the Basic Local Alignment Search Tool (BLAST). The unigenes were compared against the NCBI NR, SWISS-PROT, TrEMBL, Cdd, pfam and KOG databases with an E-value of 1e-5 and Identity of 30%. Functional annotation were operated using gene ontology terms method by Blast2GO software (GO, http://www.geneontology.org) (Ashburner et al., 2000). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were assigned to the sequences using the online KEGG Automatic Annotation Server (KAAS) (http://www.genome.jp/kegg/kaas/). This method was used to obtain KEGG Orthology (KO) assignment. The output of KEGG analysis includes KO assignments and KEGG pathways (Kanehisa, 1997; Kanehisa and Goto, 1999).

Gene expression analysis

Researchers compared the reads with unigenes using single-end mapping method by bowtie2-2.2.2 software. To compare the unigene expression level in the A and B libraries, the transcript level of each expressed unigene was calculated and normalized to the reads per kilobase of exon model per million mapped

reads (RPKM) (Mortazavi et al., 2008). Significance of differential unigene expression was determined by using General Chi-squared test and assigned P-values (<0.01). The P-values were adjusted to account for multiple testing by using the false discovery rate (FDR) and assigned error ratio Q-value (<0.05).
The unigenes with an adjusted P-value <0.01 and the absolute value of log2 >1 (expression fold change) were deemed to differently expressed, while the unigenes with an FDR-adjusted P-value <0.01 was considered statistically significant (Audic and Claverie, 1997; Reiner et al., 2003; Simonsen and Mcintyre, 2005).

RESULTS AND DISCUSSIONS

Sequence analysis and assembly

To understand the molecular bases of Zizyphus jujuba and identify the new valid genes, six libraries representing two groups of high resistant cracking fruit and sensitive cracking fruit were constructed. RNA from the two groups was used for Illumina RNA-Seq.
Each sequenced sample yielded 100bp reads from paired-end sequencing of cDNA fragments. After quality assessment and data clearance, 4.5~4.6 billion (G) reads with more than 96% Q20 bases (those with an average base quality greater than 20) were kept as high quality reads for each library and used in the later analysis (Table 1). All the valid reads above were combined to perform de novo splicing by paired-end method with Trinity software (trinityrnaseq_r20131110 version). A total of 94,984 unigenes were obtained, among which 25,287 unigenes were longer than 1kb. An overview of the assembled transcripts and unigenes was presented in Table 2. The length distributions of unigenes were shown in Figure 1. The results demonstrated the effectiveness of Illumina pyrosequencing in rapidly capturing a large portion of the transcriptome.
The distribution of gene expression levels was used to evaluate the normality of the library data. The level of gene expression was determined by calculating the number of unigenes and then normalizes to the RPKM (Mortazavi et al., 2008). As shown in Figure 2, the majority of mRNA was expressed at low levels, whereas a small proportion of mRNA was highly expressed.
The gene expression variations were analysed by compared the two libraries of resistant cracking fruits (group A) and sensitive cracking (group B). A total of 391 differently expressed genes (DEGs) including 218 up-regulated and 173 down-regulated genes were detected.

Table 1 Description of two groups of *Zizyphus jujuba* RNA-Seq libraries

Library name	Total reads	Total bases	Average reads length (bp)	Cycle Q20 (%)
A[a]	45,401,606	4,507,948,150	99.29	96.37
B[b]	46,468,222	4,606,988,891	99.14	96.04
All	91,869,828	9,114,937,041	99.22	96.20

A [a]: cracking-resistant fruit; B [b]: sensitive-cracking fruit;

Table 2 Summary of Illumina transcriptome assembly for *Zizyphus jujuba*

Library name	Total reads	N50[a]	N90[b]	Total Length	Max length	Min length	Average length
Transcript	198,993	2010	589	252,481,032	28,719	201	1268.79
Unigene	94,984	1584	302	79,036,677	28,719	201	832.11

N50 a, sorted the transcripts from long to short, then accumulated bases of transcripts in turn, when the total bases number reached half of total number of bases, the length of transcript, as well as unigenes; N90 b, counted in a similar way.

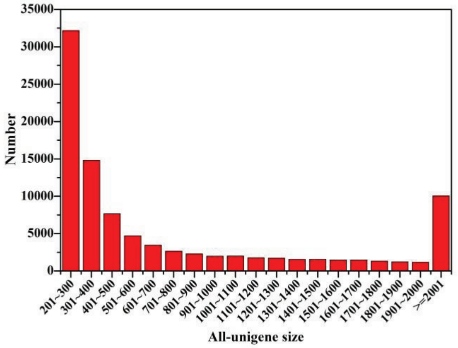


Figure 1. Length distributions of All-Unigenes

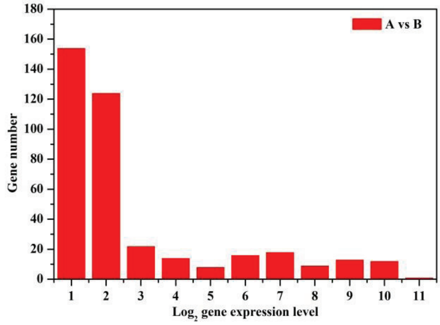


Figure 2. Distribution of gene expression levels

GO enrichment analysis of DEGs

GO enrichment analysis was the function annotations of DEGs. GO term with P-value< 0.05 were defined significantly enriched, and there were 1515 items DEGs involved in it. We mapped the sensitive-cracking higher than cracking-resistant fruit gene expression (UP.A.B) and sensitive-cracking less than cracking-resistant fruit gene expression (DOWN.A.B) DEGs to terms in GO database respectively.

The DEGs up of GO enrichment analysis are shown in Figure 3. There were 467 items UP.A.B DEGs involved in biological metabolism, mainly including metabolic processes, accounting for 30%; cellular processes, 26%; single-organism processes, 15%, and multi-organism processes, 10%; 257 items involved in cellular components, the cell accounted for 17%; cell part, 17%; other organism, 16%, and other organism part, 16%; 255 items involved in molecular function, the catalytic activity took up 60%, and binding was 27%.

The DEGs down of GO enrichment analysis are shown in Figure 4. There were 233 items DOWN.A.B DEGs involved in biological metabolism, mainly including cellular process, taking up 30%; cellular component organization or biogenesis, 15%, and metabolic process, 14%; 256 items involved in cellular component, The cell accounted for 20%; cell part, 20%; macromolecular complex, 14%; organelle, 14% and organelle part, 14%; 73 items involved in molecular function, including binding accounting for 55% and catalytic activity taking up 42%.

We compared GO enrichment analysis between UP.A.B and DOWN.A.B DEGs, there were 11 processes fully upward trend, multicellular organismal process, developmental process, reproductive process, biological adhesion, membrane, membrane part, extracellular matrix, extracellular region part, nucleic acid binding transcription factor activity, molecular function regulator and antioxidant activity. The membrane belongs to cellular component including 24 genes were all up-regulated, membrane component may have important relationship with fruit sensitive cracking.

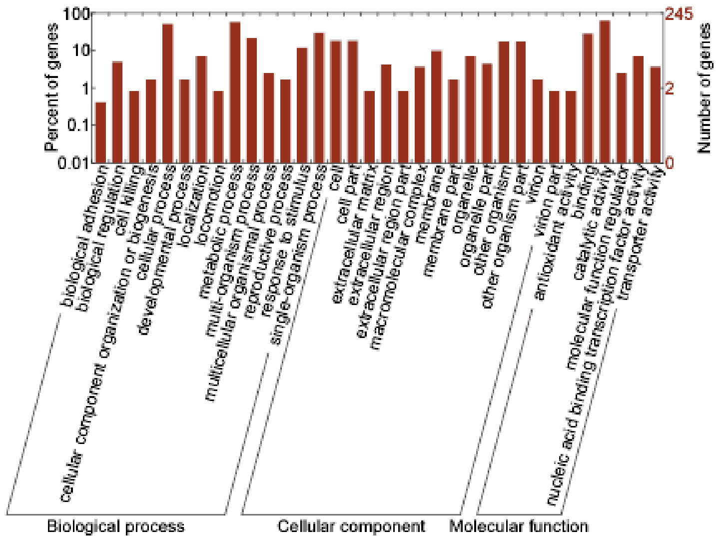


Figure 3 DEGs up of GO enrichment analysis

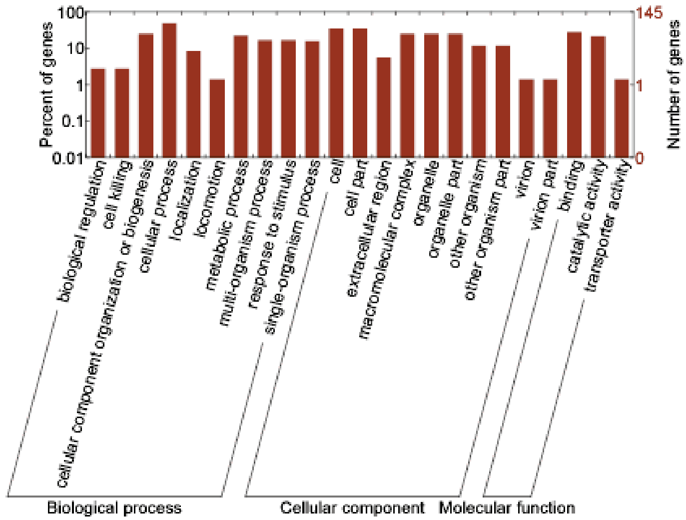


Figure 4. DEGs down of GO enrichment analysis

Pathway enrichment analysis of DEGs

KEGG pathway enrichment analysis was performed to categorize the biological functions of DEGs. (Kanehisa, 1997; Kanehisa and Goto, 1999) We mapped all the genes to terms in KEGG database.

Enrichment analysis of differentially expressed genes by KEGG is shown in Table 3. KEGG with P-value < 0.05 were defined significantly enriched, and there were 246 items DEGs involved in it. Among all the KEGG, there were 10 items significantly enriched KEGG; The DEGs participated in amino acid metabolism and lipid metabolism were more, there were 15 items and 12 items, respectively. The amino acid metabolism primarily referred to cysteine and methionine metabolism, and valine, leucine and isoleucine biosynthesis. The Lipid metabolism mainly pointed to alpha linolenic acid metabolism and steroid biosynthesis.

The experiment were expected to get the related genes of jujube easy cracking fruit or resistance cracking fruit by the KEGG enrichment analysis of DEGs, the α-linolenic acid is the basic substances of cell membrane and enzymes (SanGiovanni et al., 2005). The result of down-regulated differences in gene enrichment of metabolic pathway suggested that α-linolenic acid metabolism in cracking fruit types were weaker than in resistance cracking fruit types; therefore, α-linolenic acid metabolic pathway can be one of the research targets for cracking fruit study.

The number Ko (ko00592) in the α-linolenic acid metabolic pathway corresponding to KEGG pathway can analyse the relationship of genes and KEGG, and map the information into the pathway. The α-linolenic acid metabolism process with KEGG pathway was shown in Figure 5.

Table 3 Enrichment analysis of differentially expressed genes by KEGG

#	KEGG name term	Classification	P-value	Q-value	Number
1	Cysteine and methionine metabolism [PATH:ko00270]	Amino acid metabolism	2.89E-06	0.000890265	11
2	Flavonoid biosynthesis [PATH:ko00941]	Biosynthesis of other secondary metabolites	4.59E-11	1.41E-08	11
3	Alpha linolenic acid metabolism [PATH:ko00592]	Lipid metabolism	8.11E-07	0.000249766	8
4	Glutathione metabolism [PATH:ko00480]	Metabolism of other amino acids	0.00014206	0.043753378	8
5	Circadian rhythm - plant [PATH:ko04712]	Environmental adaptation	5.29E-06	0.001628153	4
6	ko01220	-	2.84E-05	0.008752995	4
7	Stilbenoid, diarylheptanoid and gingerol biosynthesis [PATH:ko00945]	Biosynthesis of other secondary metabolites	6.33E-05	0.019485288	4
8	Steroid biosynthesis [PATH:ko00100]	Lipid metabolism	0.00015861	0.048852201	4
9	Valine, leucine and isoleucine biosynthesis [PATH:ko00290]	Amino acid metabolism	7.25E-05	0.022328	4
10	Valine, leucine and isoleucine biosynthesis [PATH:ko00290]	Carbohydrate metabolism	0.00014	0.043056	2

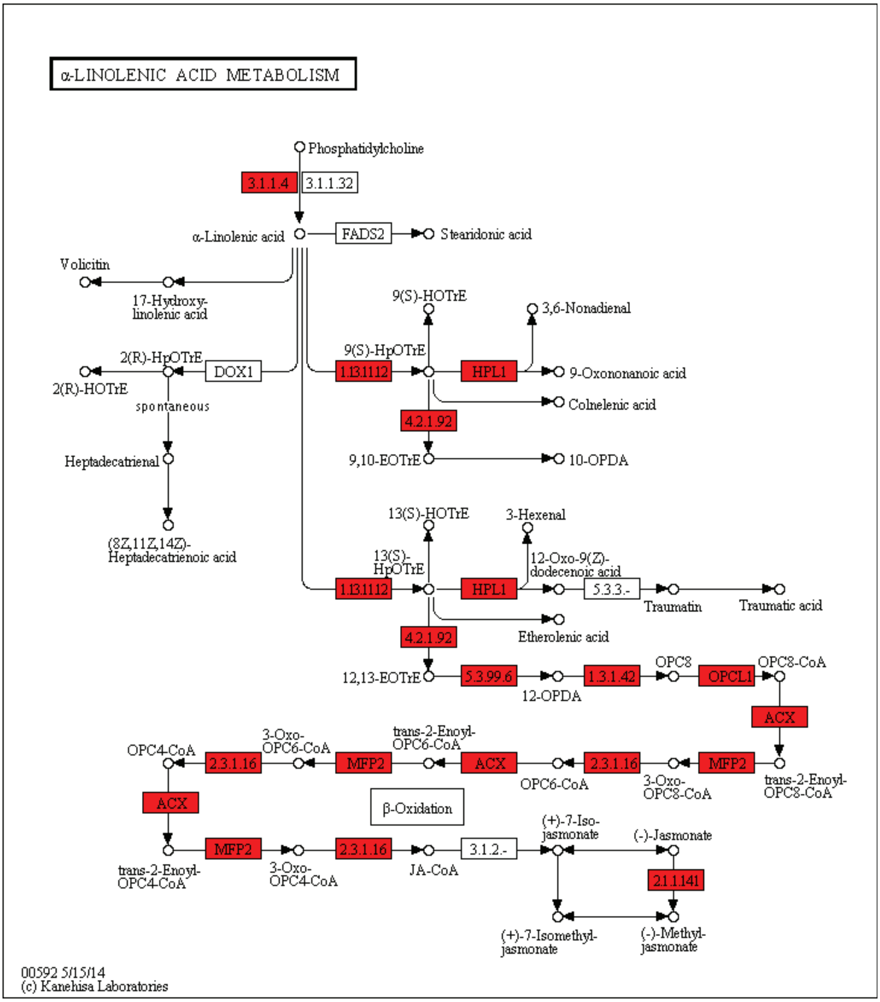


Figure 5. Alpha linolenic acid metabolism with KEGG pathway

Functional annotation of differentially expressed genes

The main gene annotations of DEGs are given in Table 4. The screened 391 items DEGs were functionally annotated, 92 items genes annotated, 45 items genes defined, and 299 items genes still not annotated. The annotated genes related to jujube cracking fruit were Aquaporin PIP, Tubulin, Calreticulin, and Calmodulin; there were 5, 6, 2 and 2 in DEGs, respectively.

Aquaporin PIP is a protein channel in the plasma membrane that facilitates water movement across the membrane, regulating the water balance inside and outside the cell, which are the down-regulated DEGs, suggesting the expression of Aquaporin PIP in resistance to cracking fruit types are higher than that in easy to cracking fruit types. Previous studies showed that

With the development of the fruit, the water in fruit was reduced, and water absorbing capacity was increased. Then when there was water in the fruit surface and dived in water potential gradient, the water would go into the fruits, thus when the turgor pressure within the fruit exceed the skin strength, it would be the cracking fruits (Wang et al., 2013).

And in the resistance to cracking fruit types, the Aquaporin PIP could promote the water transportation inside and outside cells, which reduced the turgor pressure within the fruit to a certain extent, thus reduce the risk of cracking fruits.

Tubulin is the important component of the cytoskeleton, it acts to keep the cells' shape, involved in cell division, cell movement, intracellular material transportation, and assists all kinds of organelles to complete their respective functions (Rao and Zhang, 2013). In our study, we annotated α-tubulin and β-tubulin to down-regulated DEGs, which could promote the high expression of microtubule protein gene, thus reducing the happening of cracking fruit types.

Calreticulin is one of the calcium binding proteins on the endoplasmic reticulum, with the biological function of chaperone, adjusting the steady state of Ca2+, cell adhesion and gene expression regulation (Liu, 2013), Calmodulin is one of the conservative strong regulatory proteins in the structure of biological cell by adjusting a series of enzymes to affect the plant growth, development and stress (Hoeflich and Ikura, 2002). Li (2014) screened 13 genes associated with calcium transportation from the candidate genes related to cracking fruit by DEGs. In our study, the Calreticulin and Calmodulin showed high expression in resistance to cracking fruit types, suggesting the cracking fruit had important relationship with Ca transportation and regulation.

Table 4 The main gene annotation of DEGs

Unigene	Name	Definition	Length	up/down	P-value	Q-value
comp54052_c0_seq1	PIP	AQUAPORIN PIP	1416	down	1.79E-12	2.21E-09
comp40997_c0_seq1	PIP	AQUAPORIN PIP	1508	down	6.84E-13	9.69E-10
comp16213_c0_seq1	PIP	AQUAPORIN PIP	315	down	3.99E-05	1.20E-02
comp16225_c0_seq1	PIP	AQUAPORIN PIP	315	down	3.99E-05	1.20E-02
comp45258_c1_seq1	PIP	AQUAPORIN PIP	1231	up	0.00012	0.03147
comp22094_c0_seq1	TUBA	TUBULIN ALPHA	984	down	0.00011	0.02806
comp10051_c0_seq1	TUBA	TUBULIN ALPHA	704	down	2.65E-06	1.16E-03
comp15927_c0_seq1	TUBA	TUBULIN ALPHA	984	down	4.83E-05	1.41E-02
comp49817_c0_seq1	TUBA	TUBULIN ALPHA	2035	down	4.10E-11	4.33E-08
comp49059_c0_seq1	TUBA	TUBULIN ALPHA	1978	down	2.23E-11	2.43E-08
comp36379_c0_seq1	TUBB	TUBULIN BETA	853	down	1.60E-06	7.43E-04
comp45120_c0_seq1	CALM	CALMODULIN	1210	down	0.000171	0.04298
comp45967_c0_seq1	CALM	CALMODULIN	1150	down	0.000169	0.04249
comp41077_c0_seq1	CALR	CALRETICULIN	1584	down	1.23E-06	0.00059
comp42453_c0_seq1	CALR	CALRETICULIN	1687	down	2.65E-06	0.00116

CONCLUSIONS

In summary, 5 gene fragments annotated Aquaporin PIP, 6 gene fragments annotated Tubulin, 2 gene fragments annotated Calreticulin and Calmodulin have been obtained by the differential gene analysis of two near-isogenic gene pools. All the gene fragments above were down-gene of differential genes. The result suggested that the express level of Aquaporin PIP, Tubulin, Calreticulin and Calmodulin in cracking-resistant fruit were higher than that in sensitive cracking fruit.

It was found that α-linolenic acid metabolism was related to fruit cracking of Chinese jujube according to the GO functional enrichment and KEGG enrichment analysis by building two near-isogenic pools with the same genetic background and different cracking fruit traits. It was concluded that cracking fruit process of Chinese jujube is closely linked to the cellular structure because α-linolenic acid is the basis substance of cell membrane and enzyme (SanGiovanni et al., 2005).The previous researches obtained similar results (Zhou et al., 1999; Xin et al., 2006; Liu et al., 2015).

ACKNOWLEDGEMENTS

This work was supported by the Project (14226809D, 16226313-7) from the Science and Technology Department of Hebei Province, the National Science and Technology Support Project (2013BAD14B03) and the project (2005DKA21003) from National Forest Genetic Resources Platform of China.

REFERENCES

Ashburner M., Ball C.A., Blake J. A., et al., 2000. Gene ontology: tool for the unification of biology. Nature Genetics, 25(1):25-29.

Audic S., Claverie J.M., 1997. The significance of digital gene expression profiles. Genome Research, 7(10):986-995.

Grabherr M.G., Haas B.J., Yassour M., et al., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology, 29(7):644-652.

Hoeflich K.P., Ikura M., 2002. Calmodulin in action: diversity in target recognition and activation mechanisms. Cell, 108(6):739-742(4).

Kanehisa M., 1997. A database for post-genome analysis. Trends in Genetics Tig, 13(9):375-376.

Kanehisa M., Goto, S., 1999. Kegg: kyoto encyclopedia of genes and genomes. Nucleic Acids Research, 27(1):29-34(6).

Kasai S., Hayama H., Kashimura Y., et al., 2008. Relationship between fruit cracking and expression of the expansin gene MdEXPA3 in ‘Fuji’ apples (Malusdomestica Borkh.). Scientia Horticulturae, 116(2):194-198.

Li W.C., Wu J.Y., Zhang H.N., et al., 2014. De novo assembly and characterization of pericarp transcriptome and identification of candidate genes mediating fruit cracking in Litchi chinensis Sonn. International Journal of Molecular Sciences, 15(10):17667-17685.

Liu X.M., 2013. The structure, function and evolution of the Calreticulin. Science Technology Information (16):226-226.

Liu Z.G., Lu Y.Q., Zhao J., et al., 2015. The effects of water absorbing dynamics and pericarp microstructure on fruit cracking in Chinese jujube. Journal of Plant Genetic Resources, 16(1):192-198(7).

Mao Y.M., Shen L.Y., Bi P., et al., 1998. Comparative study on cracking resistance of different Chinese jujube cultivars. The First Academic Symposium Proceedings of Dried Fruit Production and Scientific Development, 145-151.

Moctezuma E., Smith D.L., Gross K.C., 2003. Antisense suppression of a β-galactosidase gene (TB G6) in tomato increases fruit cracking. Journal of Experimental Botany, 54(390):2025-2033.

Mortazavi A., Williams B.A., Mccue K., et al., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nature Methods, 5(7):621-628.

Rao G.D., Zhang J.G., 2013. Advances of studies on plant tubulin gene. World Forestry Research. 26(3):17-20

Reiner A., Yekutieli D., Benjamini Y., 2003. Identifying differentially expressed genes using false discovery rate controlling procedures. Bioinformatics, 19(3):368-375.

Sangiovanni J.P., Chew E.Y., 2005. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. Progress in Retinal and Eye Research, 24(1):87–138.

Simonsen, K.L., Mcintyre, L.M., 2005. Implementing false discovery rate control: increasing your power. Oikos, 108(3):643–647.

Wakasa Y., Hatsuyama Y., Takahashi A., et al. 2003. Divergent expression of six expansin genes during apple fruit ontogeny. European Journal of Horticultural Science, 68(6):253-259.

Wang Y., Lu W., Li J., et al., 2006. Differential expression of two expansin genes in developing fruit of cracking-susceptible and -resistant litchi cultivars. Journal of the American Society for Horticultural Science American Society for Horticultural Science, 131(1):118-121.

Wang Z.L., Tian Y.P., Liu M.J., et al., 2011. Crack resistance and its mechanism of different Chinese jujube cultivars. Nonwood Forest Research, 29(3):74-77

Wang, B.M., Ding, G.X., Wang, X.Y., et al., 2013. Changes of histological structure and water potential of huping jujube fruit cracking. Scientia Agricultura Sinica, 46(21):4558-4568.

Xin Y.W., Ji X., Liu H., 2006. Observation and studies on peel and pulp growing characters of different crack in jujube fruit varieties. Chinese Agricultural Science Bulletin, 22(11):253-257.

Yuan Z., Lu Y.Q., Zhao J., et al., 2013. Screening and evaluation of germplasms with high resistance to fruit cracking in Ziziphus jujuba Mill. China Agriculture Science, 46(23):4968-4976.

Zhou J.Y., Mao Y.M., Shen L.Y., et al., 1998. Study on the relationship between microstructure and fruit cracking of Chinese jujube. The Academic Symposium Proceedings of Dried Fruit Production and Scientific Development, 266-267.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ISOLATION AND BIOINFORMATICS ANALYSIS OF GLUTAMYL-TRNA REDUCTASE IN CHINESE JUJUBE

ZG LIU, J ZHAO, MJ LIU

Hebei Agricultural University, 071001, Baoding, Hebei, China

Corresponding authors email: lmj1234567@aliyun.com; zhaojinbd@126.com

Abstract

Tetrapyrroles, such as chlorophyll and heme, are integral to the metabolism of all living organisms. Glutamyl-tRNA reductase (GluTR) is the first unique enzyme in the tetrapyrrole biosynthetic pathway in plants. We firstly cloned the GluTR complete sequences (1733 bp) from Chinese jujube (Ziziphus jujuba Mill., belonging to the family Rhamnaceae), named as ZjGluTR (GenBank accession no. KF530842). ZjGluTR shares high similarity (90%) to those of Malus domestica and Prunus mume through BLASTX analysis. It contains a 1659-bp ORF and encodes a predicted polypeptide of 552 amino acids, with an estimated molecular mass of 60.0 kDa and a theoretical pI of 8.55. The speculated formula of ZjGluTR is $C_{2615}H_{4318}N_{756}O_{804}S_{25}$. *ZjGluTR has a chloroplast transit peptide which contains 61 amino acids in the N terminal. The subcellular localization result showed that ZjGluTR protein exists in chloroplasts. ZjGluTR has three typical functional domains, i.e. GluTR N-terminal domain (101-259 aa), NAD(P)-binding Rossmann-fold domain (266-422 aa) and GluTR dimerization domain (425-534 aa). The molecular phylogenetic tree of GluTR indicated that the family Rhamnaceae has a close genetic relationship with the family Rosaceae. Our studies on the GluTR using molecular biology and bioinformatics approaches would play an important role in chlorophyll metabolic research of Chinese jujube.*

Key words: *Chinese jujube, GluTR, isolation, bioinformatics analysis.*

INTRODUCTION

Tetrapyrroles, such as chlorophyll and heme, are integral to the metabolism of all living organisms. The first common precursor molecule of all tetrapyrroles is derived from 5-aminolevulinic acid (ALA). It is synthesized through two distinct biosynthetic routes: Firstly, in humans, animals, fungi and the α-group of the proteobacteria, the condensation of succinyl coenzyme A and glycine with the release of CO_2 is catalyzed by ALA-synthase (Kikuchi et al., 1958; Neuberger, 1968; Avissar et al., 1989; Ferreira, 1995). Secondly, the older pathway, utilizing the C_5-skeleton of glutamate, was first discovered in plants (Beale and Castelfranco, 1973). Subsequently, the C_5-pathway was found to be common to plants, green algae, archaea and most bacteria (Schön et al., 1986; Jahn et al., 1992). In the first dedicated step, the NADPH-dependent reduction of glutamyl-tRNA to glutamate-1-semialdehyde (GSA) is catalyzed by glutamyl-tRNA reductase (GluTR) (Mayer et al., 1987; Chen et al., 1990; Jahn et al., 1992, Verkamp et al., 1992; Vothknecht et al., 1996, 1998). In the subsequent reaction, GSA is transaminated by the pyridoxal/pyridoxamine 5'-phosphate-dependent glutamate-1-seminaldehyde-2, 1-aminomutase (GSAM) to form ALA (Grimm, 1990; Jahn et al., 1991; Smith et al., 1992; Ilag and Jahn, 1992). In plants, all of the components required for such a conversion are located in the chloroplasts.

Other pathways of ALA formation in plants have been reported (Ramaswamy and Nair, 1973; Meller and Gassman, 1982), but efforts to thoroughly characterize these 'alternative ALA-forming pathways' have been unsuccessful. The existence of the C_5 pathway is widely accepted. Furthermore, lethal effect produced in some lines by antisense HEMA1 (Kumar et al., 2000) and antisense GSA (Höfgen et al., 1994) favors the C_5 pathway as a sole source for ALA biosynthesis.

Currently, studies on ALA biosynthesis in Chinese jujube (*Ziziphus jujuba* Mill.)-have not been reported. We carried out relevant research on the *GluTR* using molecular biology and bioinformatics approaches, which would provide a foundation for chlorophyll metabolic studies of Chinese jujube.

MATERIALS AND METHODS

Materials

Ziziphus jujuba Mill. 'Xingguang' was used as material to isolate total RNA. The fresh young leaves were collected, then frozen with liquid nitrogen rapidly and kept at -80 °C.

Methods

Total RNA isolation

Isolation of total RNA was carried out according to the instructions of improved CTAB method (Zhao et al., 2009). DNase I treatment was applied to remove contaminating genomic DNA. First-strand cDNA was synthesized as described by TaKaRa RNA PCR Kit (AMV) Ver.3.0 (TaKaRa).

Amplification of the full length cDNA of GluTR

Homology cloning method was used to obtain full-length cDNA of *GluTR* in Chinese jujube, the pair primers of 5' end-primer (5′-ATGGCCGTGTCGACCAGT T-3′) and 3' end-primer (5′-GAGGATGTTGCCTCTTATTC-3′) were used in this study. PCR was performed in a volume of 25 µL containing 15.5 µL of ddH_2O, 2 µL of the first strand cDNA, 2.5 µL of ExTaq DNA polymerase buffer, 2 µL of $MgCl_2$, 0.5 µL of dNTPs, 0.5 µL of ExTaq DNA polymerase, and 1 µL of each primer. PCR were optimized to consist with the following parameters, i.e. denaturation of 5 min at 95 °C; 35 cycles of 30 s at 94 °C, 60 s at 55 °C and 90 s at 72 °C; extension at 72 °C for 10 min. PCR products were separated on 1% agarose gels. Amplified fragment was cloned into pMD-19T vector and sequenced by Beijing ZhongKe XiLin Biotechnology CO., Ltd.

Analysis of cDNA and protein sequences

cDNA sequence was analyzed using BLAST (http://www. http://blast.ncbi.nlm. nih.gov/) and the ORF FINDER (http://www.ncbi.nlm.nih.gov/ gorf/gorf.html). The protein sequence was analyzed by Protparam (http://expasy.org/ tools/protparam.html), WoLF PSORT (http://wolfpsort.seq.cbrc.jp), ChloroP Server 1.1 (http://www.cbs.dtu.dk/services/ ChloroP/), Pfam (http://pfam.sanger.ac.uk) and SWISS-MODEL Workspace (http://www.expasy.ch/swissmod/ SWISS-MODEL.html). Homology tree was educed according to MEGA 6 using neighbor-joining method.

RESULTS AND DISCUSSIONS

Cloning of the full-length cDNA of *ZjGluTR*

Approximately 1750-bp fragment was identified by homology cloning method (Figure 1). The fragment was cloned into a cloning vector, and sequenced subsequently. A 1733-bp expressed sequence tags (EST) was obtained after removing vector and adapter sequences. We named the sequence as *ZjGluTR* (GenBank accession no. KF530842). BLASTX analysis showed that the 1733-bp sequence shared high similarity (90%) to those of *Malus domestica* (XP008378624.1) and *Prunus mume* (XP008219178.1).

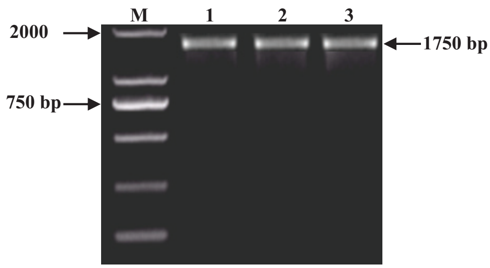


Figure 1. The PCR result of *ZjGluTR* by homologous cloning. Note: line 1, 2 and 3 were three replication

Protein sequence analysis and characterization of ZjGluTR

The full-length cDNA sequence of *ZjGluTR*, containing a 1659-bp ORF, encoded a predicted polypeptide of 552 amino acids, with an estimated molecular mass of 60.0 kDa and a theoretical pI of 8.55. The speculated formula of protein was $C_{2615}H_{4318}N_{756}O_{804}S_{25}$.

The results predicted from Search Pfam showed that ZjGluTR contained three functional domains, including GluTR N-terminal domain, Shikimate/quinate 5-dehydrogenase domain and GluTR dimerisation domain. GluTR N-terminal domain and GluTR dimerisation domain were typical characteristics of GluTR.

Relevant literature reported that the precursor protein of Glutamyl-tRNA reductase has a transit peptide in the N terminal. Therefore, we carried out transit peptide prediction for the ZjGluTR. The results showed that the ZjGluTR has a chloroplast transit peptide which contains 61 amino acids in the N terminal. At the same time, WoLF PSORT was used to predict the ZjGluTR subcellular localization. The result showed that ZjGluTR protein exists in plant chloroplasts with the identity of 79%. This result was consistent with the ZjGluTR chloroplast transit peptide prediction.

Alignment result by homology modeling showed that ZjGluTR has three typical functional domains (Figure 2), such as GluTR N-terminal domain (101-259 aa), NAD(P)-binding Rossmann-fold domains (266-422 aa) and GluTR dimerization domain (425-534 aa). The results were consistent with Schubert's reports that each GluTR monomer consists of three distinct domains (Schubert et al., 2002). In addition, Shikimate/quinate 5-dehydrogenase domain (262-414 aa) was also predicted, which was not the typical characteristic of GluTR. Meanwhile, the tertiary structure of ZjGluTR was constructed by SWISS-MODEL Workspace (Figure 3b), which has the same tertiary structure as *Methanopyrus kandleri* GluTR (Figure 3a) (Schubert et al., 2002).

```
IPR000343: Glutamyl-tRNA reductase, Family
  TIGR01035: 104 - 533  hemA: glutamyl-tRNA reductase

IPR000343: Glutamyl-tRNA reductase, Family
  SSF69742: 101 - 259   Glutamyl tRNA-reductase catalytic, N-terminal domain

IPR006151: Shikimate/quinate 5-dehydrogenase, Domain
  PF01488: 262 - 414    Shikimate_DH

noIPR: unintegrated, unintegrated
  SSF51735: 266 - 422   NAD(P)-binding Rossmann-fold domains

noIPR: unintegrated, unintegrated
  SSF69075: 425 - 534   Glutamyl tRNA-reductase dimerization domain
```

Figure 2. Alignment results of *ZjGluTR* homology modeling

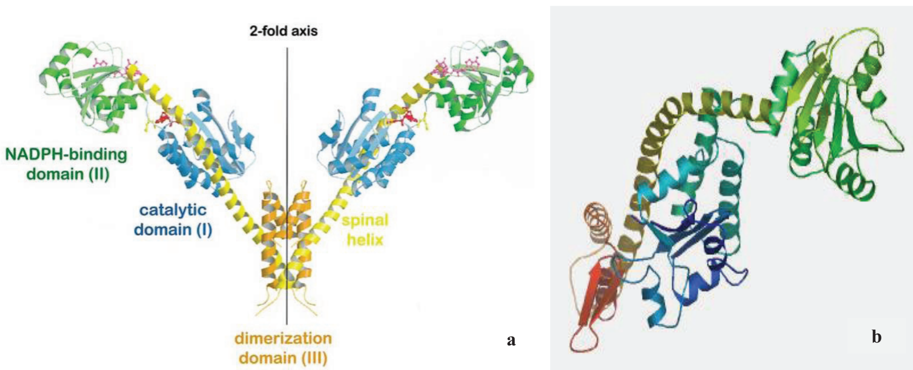


Figure 3. A schematic diagram of the *Methanopyrus kandleri GluTR* dimer viewed perpendicular (a) (Schubert et al., 2002) and the tertiary structure of *ZjGluTR* (b)

The phylogenetic tree of GluTR

The molecular phylogenetic tree of ZjGluTR and other 11 plant species was constructed by MEGA 6 (Figure 4). This tree showed that GluTR of *Z. jujuba* (belonging to the family Rhmnaceae) was firstly clustered with that of *Malus domestica* and *Prunus mume* (the family Rosaceae) which indicated that ZjGluTR should have a closely genetic relationship with that of Rosaceae.

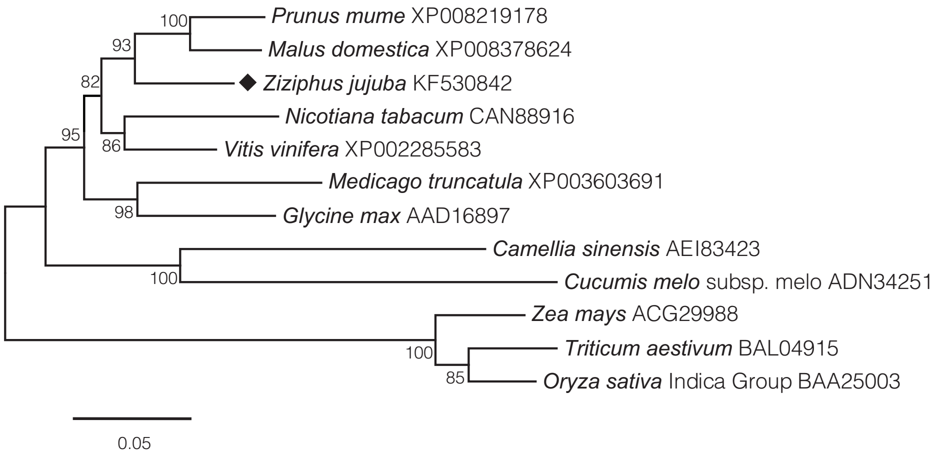


Figure 4. The phylogenetic tree of *ZjGluTR* and other 11 plant species

CONCLUSIONS

GluTR is the first committed enzyme in plant tetrapyrrole biosynthesis and is likely to be involved in the control of this metabolic pathway. The tetrapyrrole biosynthetic pathway provides the vital cofactors and pigments for photoautotrophic growth (chlorophyll) (Czarnecki et al., 2011). Some of the transgenic studies showed that plant chlorophyll deficiency, ranging from patchy yellow to total yellow. Moreover, the plants that completely lacked chlorophyll failed to survive under the growth conditions (Kumar et al., 2000; Höfgen et al., 1994). These observations suggest that suppression of the enzymes of the C_5 pathway affect the growth of the plant. Therefore, studies on the regulated synthesis of tetrapyrroles, including heme and chlorophyll, are important. Thus, cloning and bioinformatics analysis of *ZjGluTR* is very helpful for molecular biology research of chlorophyll synthesis in Chinese jujube.

REFERENCES

Vissar Y J, Ormerod J G, Beale S I, 1989. Distribution of δ-aminolevulinic acid biosynthetic pathways among phototrophic bacterial groups. Archives of microbiology, 151(6): 513-519.

Beale S I, Castelfranco P A, 1973. ^{14}C incorporation from exogenous compounds into δ-aminolevulinic acid by greening cucumber cotyledons. Biochemical and biophysical research communications, 52(1): 143-149.

Chen M W, Jahn D, O'Neill G P et al., 1990. Purification of the glutamyl-tRNA reductase from Chlamydomonas reinhardtii involved in delta-aminolevulinic acid formation during chlorophyll biosynthesis. Journal of Biological Chemistry, 265(7): 4058-4063.

Czarnecki O, Hedtke B, Melzer M et al., 2011. An Arabidopsis GluTR binding protein mediates spatial separation of 5-aminolevulinic acid synthesis in chloroplasts. The Plant Cell Online, 23(12): 4476-4491.

Ferreira G C, 1995. Heme biosynthesis: biochemistry, molecular biology, and relationship to disease. Journal of bioenergetics and biomembranes, 27(2): 147-150.

Grimm B, 1990. Primary structure of a key enzyme in plant tetrapyrrole synthesis: glutamate 1-semialdehyde aminotransferase. Proceedings of the National Academy of Sciences, 87(11): 4169-4173.

Höfgen R, Axelsen K B, Kannangara C G et al., 1994. A visible marker for antisense mRNA expression in plants: inhibition of chlorophyll synthesis with a glutamate-1-semialdehyde aminotransferase antisense gene. Proceedings of the National Academy of Sciences, 91(5): 1726-1730.

Ilag L L, Jahn D, 1992. Activity and spectroscopic properties of the *Escherichia coli* glutamate 1-semialdehyde aminotransferase and the putative active site mutant K265R. Biochemistry, 31(31): 7143-7151.

Jahn D, Chen M W, Söll D, 1991. Purification and functional characterization of glutamate-1-semialdehyde aminotransferase from Chlamydomonas reinhardtii. Journal of Biological Chemistry, 266(1): 161-167.

Jahn D, Verkamp E, 1992. Glutamyl-transfer RNA: a precursor of heme and chlorophyll biosynthesis. Trends in biochemical sciences, 17(6): 215-218.

Kikuchi G, Kumar A, Talmage P et al. The enzymatic synthesis of δ-aminolevulinic acid. Journal of Biological Chemistry, 1958, 233(5): 1214-1219.

Kumar A M, Söll D, 2000. Antisense HEMA1 RNA expression inhibits heme and chlorophyll biosynthesis in Arabidopsis. Plant physiology, 122(1): 49-56.

Mayer S M, Beale S I, Weinstein J D, 1987. Enzymatic conversion of glutamate to delta-aminolevulinic acid in soluble extracts of *Euglena gracilis*. Journal of Biological Chemistry, 262(26): 12541-12549.

Meller E, Gassman M L, 1982. Biosynthesis of 5-aminolevulinic acid: two pathways in higher plants. Plant Science Letters, 26(1): 23-29.

Neuberger A, 1968. Biochemical basis and regulatory mechanisms of porphyrin synthesis. Proceedings of the Royal Society of Medicine, 61(2): 191-193.

Ramaswamy N K, Nair P M, 1973. δ-Aminolevulinic acid synthetase from cold-stored potatoes.

Biochimica et Biophysica Acta (BBA)-Enzymology, 293(1): 269-277.

Schön A, Krupp G, Gough S, et al., 1986. The RNA required in the first step of chlorophyll biosynthesis is a chloroplast glutamate tRNA. Nature, 322: 281-284.

Schubert W D, Moser J, Schauer S et al., 2002. Structure and function of glutamyl-tRNA reductase, the first enzyme of tetrapyrrole biosynthesis in plants and prokaryotes. Photosynthesis research, 74(2): 205-215.

Smith M A, Kannangara C G, Grimm B, 1992. Glutamate 1-semialdehyde aminotransferase: anomalous enantiomeric reaction and enzyme mechanism. Biochemistry, 31(45): 11249-11254.

Verkamp E, Jahn M, Jahn D, et al., 1992. Glutamyl-tRNA reductase from *Escherichia coli* and *Synechocystis* 6803. Gene structure and expression. Journal of Biological Chemistry, 267(12): 8275-8280.

Vothknecht U C, Kannangara C G, Von Wettstein D, 1996. Expression of catalytically active barley glutamyl tRNAGlu reductase in *Escherichia coli* as a fusion protein with glutathione S-transferase. Proceedings of the National Academy of Sciences, 93(17): 9287-9291.

Vothknecht U C, Kannangara C G, von Wettstein D, 1998. Barley glutamyl $tRNA^{Glu}$ reductase: Mutations affecting haem inhibition and enzyme activity. Phytochemistry, 47(4): 513-519.

Zhao J, Liu Z C, Dai L et al., 2009. Isolation of Total RNA for Different Organs and Tissues of *Ziziphus jujuba* Mill. Journal of Plant Genetic Resources, 111-117.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECT OF CITRIC ACID AND TREATMENTS ON PRESERVATION OF ASCORBIC ACID IN PROCESSING OF CHINESE JUJUBE JUICE

Zhihui ZHAO, Sujuan GONG, Lili WANG, Mengjun LIU

Jujube Research Center, Hebei Agricultural University,
289 Lingyusi Street, Baoding, China

Corresponding author email: lmj1234567@aliyun.com

Abstract

Effects of citric acid and treatments on preservation of ascorbic acid in processing of Chinese jujube juice were investigated. In our research, we chose for testing Ziziphus jujuba Mill. 'Zanhuangdazao'. Before crushing the jujube fruits, we tried three trials including 1% salt solution treating the jujube fruit about 0.5 h, 5% sugar solution treating the jujube fruit less 1h and putting the jujube fruit in 60℃ water about 5 minutes, and the preserving rate of ascorbic acid were 68.2%, 68.9% and 121.9% respectively. During the crushing, we put the 0.10% citric acid into the mix of water and jujube pulps, this method could raise the preserving rate of ascorbic acid to 79% compared to the contrast (29.9%). The study of pasteurization, boiling water sterilization and autoclaving indicated that pasteurization was the best sterilization mean with the preserving rate of ascorbic acid to 44.2%, boiling water sterilization was the second with the preserving rate of ascorbic acid to 44.1%, and the autoclaving was far worse than the others with the preserving rate of ascorbic acid only to 19.4%.

Key words*: chinese jujube juice, citric acid, ascorbic acid, preserving rate, high performance liquid chromatography.*

INTRODUCTION

Chinese jujube (*Ziziphus jujuba* Mill.), a native plant of China, belongs to the genus *Ziziphus* (Rhamnaceae) and is a medicinal plant of China (Liu et al., 2009), is used as a significantly traditional Chinese medicine and invigorant(Li et al., 2009). It has crucial activities just as nourishing blood, fitting brain, activities of sedation, calm the nerves, antitumor effect, anti-aging and boost immunity(Sweetman, 2005; Gioia et al.,2008; Vidovic et al.,2008;Yu,2008;Yang et al.,2008.) . The contents of cAMP, flavones, vitamins, dietary fiber, polysaccharide, triterpene acid are remarkable (Guil-Guerrero et al., 2004; San et al., 2010; Zhao et al., 2010) in the jujube fruits, particularly the ascorbic acid. Its average content reaches to 300-600 mg/100 g based on fresh weight, even some can reach to 800-900 mg/100 g. Especially the white maturing fruit involves considerable amounts of vitamins (Gao et al., 2011). The content of ascorbic acid is higher than that in the other fruits, which known as their highly ascorbic acid, such as kiwi fruit, orange, lemon, and so on. Nowadays, there are many kinds of jujube products, such as dried jujube, honeyed jujube, jujube wine, jujube vinegar, jujube tea, jujube juice, etc.. Ascorbic acid is generally recognized as safe (GRAS) when used in accordance with Good Manufacturing Practices (21CFR182. 2000), is relatively inexpensive, and is widely recognized by consumers as a beneficial nutrient (vitamin C) (Kokkinidou et al., 2014). Vitamin C is a very important vitamin for human nutrition that is supplied by fruits and vegetables. Ascorbic acid is the main biologically active form of vitamin C. As a potent antioxidant, it has the capacity to eliminate several different free radicals (Scherera et al., 2012). However, many nutrients will be destroyed in the processing of products, especially ascorbic acid (Sheetal et al., 2013; Mapson et al., 1958; Maria et al., 2013). The ascorbic acid is so active and easily be broken by external condition just like heat, oxygen, light ray and pH. So, the content of ascorbic acid is low in the current jujube products. Therefore, it is very necessary to research a new method to protect ascorbic acid. In our research, we chose *Ziziphus jujuba* Mill. 'Zanhuangdazao' as the material and investigated the effect of citric acid and treatments on preservation of ascorbic acid.

MATERIALS AND METHODS

Ziziphus jujuba Mill. 'Zanhuangdazao' gained from Zanhuang, Hebei province was selected. The jujube fruits were washed under running water to remove the adhering mud particles followed by double distilled water and drained completely. Then we divided the fruits into three groups. Immerged the first group fruits into 1%, 2% and 3% salt solution for 0.5 h, 1.5 h, 2.5 h, 3.5 h, 4.5 h. Put the second group into 5%, 10% and 15% sugar solution for 0.5 h, 1.5 h, 2.5 h, 3.5 h, 4.5 h. At last treated the others by 60°C water for 5 min, 10 min, 15 min, 20 min, 25 min. The contents of ascorbic acid in jujube fruits were detected by high performance liquid chromatographic methods. To prevent the loss of vitamins, all operations were performed in the absence of direct sun light, using amber glassware. All tests were performed in triplicate.

During the crushing, we put the 0.01%, 0.05% and 0.10% citric acid into the mix of water and jujube pulps, then determined the content of ascorbic acid in the juice and metered the preservation rate of ascorbic acid. All tests were performed in triplicate.

To protect ascorbic acid in the jujube juice, we investigated three kinds of sterilization methods, just like pasteurization (65°C, 30 min), boiling water sterilization (100°C, 15 min) and autoclaving (121°C, 10 min).

The results were compared by one-way analysis of variance (ANOVA) and Duncan's test was carried out to identify significant differences between the mean values. All analyses were performed using the software SPSS 17.0 and differences at $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSIONS

Effect of preprocessing on preserve rate of ascorbic acid

As can be observed in Figure 1, after 1% salt solution treating the jujube fruit about 0.5 h, the preserve rate of ascorbic acid was 68.2% which higher than the contrast (66.8%).

As Figure 2 showed, 5% sugar solution treating the jujube fruit less 1 h, the preserve rate of ascorbic acid was 68.9%, it was also better than the contrast (66.8%).

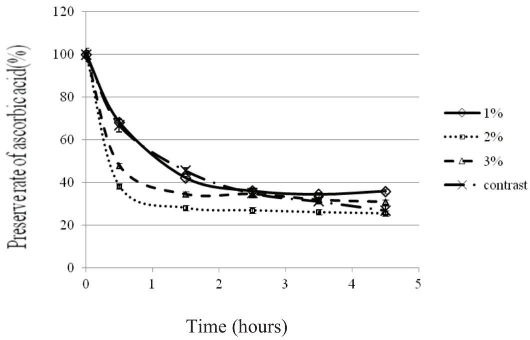


Figure 1. Influence of salt solution to the preserving rate of ascorbic acid

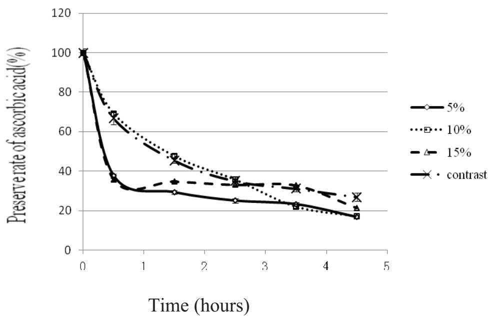


Figure 2. Influence of sugar solution to the preserving rate of ascorbic acid

Figure 3 indicated that putting the jujube fruit in 60°C water about 5 minutes, the preserving rate of ascorbic acid reached to 121.9% which obviously higher than the contrast (66.8%), this will have practical value to the processing industries of jujube fruits.

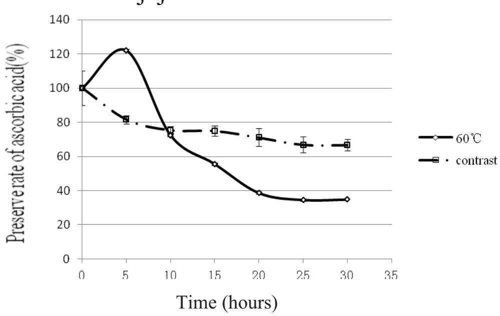


Figure 3. Influence of softening treatment to the preserving rate of ascorbic acid

Effect of citric acid on preservation of ascorbic acid

As can be observed in Figure 4, during the crushing, putting into 0.01% and 0.05% citric acid could rise the preserve rate of ascorbic acid to 43% and 46% respectively, compared by the contrast (29%), especially after the 0.10% citric acid treatment, the preserve rate of ascorbic acid reach to 79%, much higher than he contrast (29%).

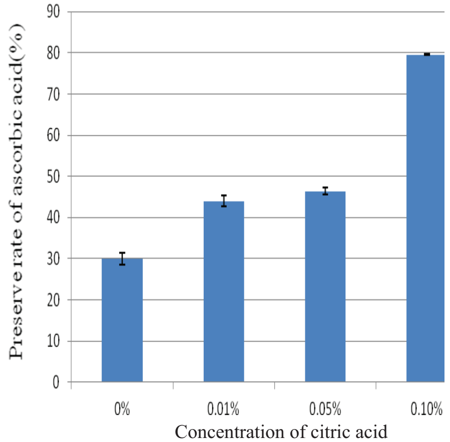


Figure. 4 Influence of citric acid on the preserving rate of ascorbic acid

Influence of the sterilization conditions on preservation of ascorbic acid

The results of the sterilization conditions (Table 1) indicated that the result of pasteurization and boiling water sterilization were ideal with the content of ascorbic acid were 226.5 mg/100 g and 225.9 mg/100 g, the preserve rate of ascorbic acid reached to 44.2% and 44.1%. However the result of autoclaving was less than the others.

Table 1 comparison of sterilization of jujube juice

Sterilization conditions	Content of ascorbic acid (mg/100g)
Pasteurization (65°C, 30 min)	226.5±3.8a
boiling water sterilization (100°C, 15 min)	225.9±1.9a
Autoclaving (121°C, 10 min)	99.4±3.6b

Each value is expressed as means ± standard deviation of n=3 determinations;
Different letters within a column (a-d) denote significant differences ($p<0.05$) between cultivars.

CONCLUSIONS

Putting the jujube fruit in 60°C water about 5 minutes before crushing fruits could keep the preserving rate of ascorbic acid to 121.9%. During the crushing, putting the 0.10% citric acid into the mix of water and jujube pulps could raise the preserving rate of ascorbic acid to 79% compared to the contrast (29.9%). These methods were proved to be useful technique to increase stability of ascorbic acid.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Hebei Provincial Department of Education (QN2015232), Top-notch Talent Support Project of Hebei province and Young Academic Leader of Hebei Agricultural University.

REFERENCES

Liu MJ, Wang M., 2009. Germplasm Resources of Chinese Jujube, Forestry Publishing. Beijing, China.

Li JW, Ding SD, Ding XL., 2005. Comparison of antioxidant capacities of extracts from five cultivars of Chinese jujube, Process Biochemistry, 40, 3607-3613.

Gao YR, Zhao ZH and Liu MJ., 2011. Determination of ascorbic acid and dehydroascorbic acid in Chinese jujube by high performance liquid chromatography, Asian Journal of Chemistry, 23, 3989.

Gioia M.G., Andreatta P., Boschetti S., Gatti R., 2008. Development and validation of a liquid chromatographic method for the determination of ascorbic acid, dehydroascorbic acid and acetaminophen in pharmaceuticals. Journal of Pharmaceutical and Biomedical Analysis, 48, 331-339.

Guil-Guerrero J.L, Delgado A.D, Matallana M.C, GONZÁLEZ, Isasa M.E. Torija., 2004. Fatty acids and carotenes in some Ber (*Ziziphus jujuba* Mill.) varieties, Plant Foods for Human Nutrition (Formerly Qualitas Plantarum), 59, 23-27.

Kokkinidou S. Floros J. D. LaBorde L. F., 2014. Kinetics of the thermal degradation of patulin in the presence of ascorbic acid. J Food Sci, 79(1): 108-114.

Mapson L.W., Tomalin A. W., 1958. Preservation of ascorbic acid in rose hips during storage. J. Sci. Food Agric, 9: 424–430.

Maria D.C. Antunes, Daniela Rodrigues, Vasilios Pantazis, et al., 2013. Nutritional Quality Changes of Fresh-cut Tomato during Shelf Life. Food Sci. Biotechnol, 22(5): 1229-1236.

San B, Yildirim A N., 2010. Phenolic, alpha-tocopherol, beta-carotene and fatty acid composition of four promising jujube (*Ziziphus jujuba* Mill.) selections. Journal of Food Composition and Analysis, 23, 706-710.

Scherera R., Rybkaa A. C. P., Ballus C.A, et al., 2012. Validation of a HPLC method for simultaneous determination of main organic acids in fruits and juices. Food Chemistry, 135: 150–154.

Sheetal Gupta, Gowri B. S., Jyothi Laksh mi A., Prakash J., 2013. Retention of nutrients in green leafy

vegetables on dehydration. J Food Sci Technol, 50(5):918–925.
Sweetman SC, 2005. Dose adjustment in renal impair-rment: Response from Martindale: the Complete Drug Reference, 331-292.
Vidovic S, Stojanovic B, Veljkovic J, Prazic-Arsic L, Roglic G, Manojlovic D., 2008. Simultaneous determination of some water-soluble vitamins and preservatives in multivitamin syrup by validated stability-indicating high-performance liquid chromatography method. Journal of Chromatography, 1202(2), 155-162.
Yang SM, Yang XM, 2008. Research trends and treatment prospects of ascorbic acid. China Medical Herald, 28(5): 11- 13.
Yu CL, 2008. Research and new influence of ascorbic acid. Jilin Medical Journal, 29(21): 145- 147.
Zhao ZH, Liu MJ, 2010. Study of properties of crude water soluble polysaccharides from *Ziziphus jujuba* Mill. “Jinsixiaozao”, Journal of Agricultural University of Hebei, 33, 58-04.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE PROPAGATION OF TWO RED AND BLACK CURRANT VARIETIES BY HARDWOOD CUTTINGS COMBINING SUBSTRATE AND ROOTING STIMULATORS

Adrian ASĂNICĂ[1], Valerica TUDOR[1], Dorin SUMEDREA[2], Răzvan Ionuț TEODORESCU[1], Adrian Peticilă[1], Alexandru IACOB[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania
[2]Research Institute for Fruit Growing Pitesti, 402 Mărului Street, Mărăcineni 117450, Romania

Corresponding author email: adrian.asanica@horticultura-bucuresti.ro

Abstract

The planting material production is a particularly attractive segment of fruit growing field, both through the possibility of maximizing profits on small lands and because of the increased interest manifested by growers in the horticultural sector, especially in berries. The currant is specie that manages to propagate more easily than other berry shrubs, but rooting capacity vary from species to species and depending on different climatic and technological factors. The present paper supports the nursery activity by developing a complex experimental module that identifies the best solutions for black and red currant cuttings as a way of vegetative propagation. The thickness of the cuttings, rooting substrates and rooting hormonal stimulants (obtained in laboratory and commercial products) are taken into account. Two varieties of currants were used: 'Tinker' cultivar for black currant and 'Elite' cultivar for red currant. The rooting percentage of cuttings was very similar for the black and currant varieties (around 77%). For both currants, the thinner cuttings gave the best results in terms of rooted cuttings percentage. Perlite + sand substrate achieved the highest rooting percentage of the cuttings but with the lowest vegetative growths. Among the stimulators used for rooting, the IBA hormone (regardless of the concentration used) achieved the best percentages of cuttings rooted in both currant varieties. IBA 1000 ppm gave the best results on the length of the shoots on both currant varieties. Peat + sand positively influenced the root volume in the red currant and perlite + peat mixture for black currant. The volume of the root system in the red currant was approximately 2 times larger than the black currant. IBA 1000 and IBA 1500 conducted to the most extensive radicular system to both currant varieties.

Key words*: rooting hormons, cuttings thickness, 'Elite','Tinker', root system volume.*

INTRODUCTION

In horticulture, vegetative propagation is widely used to multiply elite plants obtained in breeding or selected from natural populations (Hartman et al., 1990). Propagation of currant's varieties is still one of the most cost-effective methods for vegetative regeneration as any other woody species (Koyuncu and Senel, 2003; De Klerk et al., 1999). It reclaims cuttings from the previous season's growth (minimum one year old) harvested during the dormant stage from the mother plant (Sandor, 2007).

Induction of roots is a process regulated by timing (Szecsko et al., 2002), environmental and endogenous factors including hormones. Auxin is one of the major endogenous hormones involved in the adventitious rooting process (Wiesman et al, 1988; Pop et al., 2011).

Well known sources of growth hormones for rooting of cuttings are the IBA (indole-3-butyric acid), IAA (indole-3-acetic acid) and NAA (α-naphthalene acetic acid) in different concentrations (Khudhur et al., 2015; Uniyal et al., 1993), together with a lot of commercial products nowadays used as root promoters (solutions or powders).

In a previous research, Pandey et al. (2011) concluded that IBA treatment had shown not only an improvement of the percent rooting at *Ginkgo biloba* L. but also a positive effect on the subsequent growth and survival rate of the plantlets.

IBA has recorded the highest recognition among all auxins used for rooting.

Treatments of the currants hardwood cuttings with furostanol glycosides (Caulet et al., 2012), for one hour, leaded to an increasing of roots number and contributed to obtain more qualitative black currant cuttings of Deea, Abanos and Ronix varieties in comparison with Radistim 2% (commercial rooting stimulator product).

Combined and softwood cuttings have been experienced for several currant varieties by Siksnianas and Sasnauskas (2006) using IAA, NAA and ascorbic acid as rooting growth stimulators. Substrate used was 2 parts of peat and 1 part of sand and artificial mist was provided. Results showed that for cultivars of black currant, rooting of combined cuttings was higher and the NAA 25 g/l-1 offered the highest quality of rooting.

There is always a need for improvement and in this regard, the current study was conducted to determine the most suitable way to propagate two of the most cultivated varieties of black and red currant by hardwood cuttings. Thickness of the cuttings, rooting substrate and stimulator concentrations influence has been took into consideration.

MATERIALS AND METHODS

The experiment was established in the Vegetation House of the University of Agronomic Sciences and Veterinary Medicine of Bucharest and was carried out between February and June of 2016.

The biological material consists of two varieties of currant: ‘Elite’ (red currant) and ‘Tinker’ (black currant).

The cuttings were made using annual dormand branches harvested from mother plants located in the experimental field of the Faculty of Horticulture, Bucharest.

Two categories of cuttings have been executed (figure 1):

- for red currant: thin cuttings (0.26-0.35 mm) and thick cuttings (0.36-0.78 mm)
- for black currant: thin cuttings (0.3-0.48 mm) and thick cuttings (0.49-1.89 mm)

The length of the cuttings was of 15 cm with minimum 3 nodes.

Cuttings were afterwards treated with different rooting hormones and concentrations as follows:

- IBA 500 ppm (solution)
- IBA 1000 pm (solution)
- IBA 1500 ppm (solution)
- Razormin (solution of aminiacids, macroelements and microelelments)
- Rhizopon AA1% (powder, IAA 1%)

Figure 1. Cuttings of red (left) and black currant (right) made with different thickness

Except the control, all the cuttings were submerged in the hormonal solutions for 5 minutes with the basal part or covered with powder in the situation of Rhizopon product.

Rooting substrate was represented by equal share of the following parts (figure 2).

Figure 2. Rooting substrate variants:
top Peat + Perlite = 1:1
middle Peat + Sand = 1:1
bottom Perlite + Sand = 1:1

At the end, 720 cuttings resulted: 360 of red currant and 360 of black currant. Half of them (360 cuttings) were in the first thickness category and the other half in the second one. In every pot it were placed 10 cuttings of currant according to the experimental model A number of 72 pots with the capacity of 3 liter each were customized (figure 3).

Figure 3. Experimental module of currant cuttings grouped by substrate, thickness and hormonal treatment (4.04.2016)

On 7th of June, 2016, all the cuttings were removed from the pots and several determinations and observations were performed: rooting percentage, vegetative growth of the rooted cuttings, root system volume.

All data were processed by analyze of variance and Duncan's multiple range test at confidence level of 95% ($P \leq 0.05$) using XLSTAT software.

RESULTS AND DISCUSSIONS

Five months after the experiment start, the entire plot was disassembled, taking each variant one by one in careful observation and gathering data related to the rooting success and overall cuttings quality.

In this respect, we remarked very small differences concerning the rooting ratio of the two currant species. As an overall percentage, ‘Elite’ variety recorded 77.22% rooting percentage; very close to the black currants ‘Tinker’ ones of 77.36% (table 1).

Depending on the cuttings thickness, the rooting percentages were quite variable; significantly differences have been registered at red currant thinner cuttings for control and variants treated with Razormin.

At black currant, the cuttings thickness did not influence much the rooting percentage, only 10.84% difference was noticed between thicker and thinner cuttings.

Comparing the two species on this issue, only few rooting percentages (3.33%) more have been registered between thicker black currant cuttings than red currants cuttings.

Same observation was made in the case of thinner cuttings (3.61%) but in favour of red currants.

The rooting substrate played a very important role in the rooting percentage of the cuttings, at the end of the experiment, the highest values been revealed for the perlite with sand mixture (more than 80%).

We find a relatively close hierarchy between substrate types, the second place being peat + perlite which averaged 77.08%. Peat + sand had a slightly weaker effect, with lower black currant (62.92%) and almost 10% lower than ‘Elite’ red currant variety.

Table 1. Rooting percentage of currant cuttings depending on cultivar, thickness of cutting, substrate and hormones

Red currant	%	Hormone/Product					Control	Average
Cutting type	Substrate	IBA 500	IBA 1000	IBA 1500	Razormin	Rhizopon		
Thin	Peat + Perlite	100	90	60	0	80	80	**68.33a**
	Peat + Sand	100	60	60	60	60	0	**56.67a**
	Perlite + Sand	90	100	100	20	90	80	**80.00a**
	Average	**96.67a**	**83.33a**	**73.33ab**	**26.67b**	**76.67ab**	**53.33ab**	**68.33a**
Thick	Peat + Perlite	70	100	90	30	100	80	**78.33a**
	Peat + Sand	80	100	80	100	70	90	**86.67a**
	Perlite + Sand	100	90	100	80	90	100	**93.33a**
	Average	**83.33a**	**96.67a**	**90.00a**	**70.00a**	**86.67a**	**90.00a**	**86.11a**
	Overall Average (%)	***90.00a***	***90.00a***	***81.67a***	***48.33a***	***81.67a***	***71.67a***	***77.22a***
Black currant	%	Hormone/Product					Control	Average
Cutting type	Substrate	IBA 500	IBA 1000	IBA 1500	Razormin	Rhizopon		
Thin	Peat + Perlite	80	80	100	80	60	20	**70.00ab**
	Peat + Sand	60	60	60	55	60	50	**57.50b**
	Perlite + Sand	100	100	90	60	80	100	**88.33a**
	Average	**80.00a**	**80.00a**	**83.33a**	**65.00a**	**66.67a**	**56.67a**	**71.94ab**
Thick	Peat + Perlite	80	90	100	90	90	100	**91.67a**
	Peat + Sand	90	90	60	10	70	90	**68.33b**
	Perlite + Sand	70	90	90	90	90	100	**88.33ab**
	Average	**80.00a**	**90.00a**	**83.33a**	**63.33a**	**83.33a**	**96.67a**	**82.78ab**
	Overall Average (%)	***80.00a***	***85.00a***	***83.33a***	***64.17a***	***75.00a***	***76.67a***	***77.36a***

*Duncan’s multiple range test ($P \leq 0.05$)

Regarding the effect of rooting stimulants on the percentage of root formation, we noted a fairly good influence of the IBA hormone regardless of the concentration in which it was used. A negative response was also noticed at Razormin, which practically had an antagonistic effect, the rooting percentages being lower for both species but more evident in the red bark (48.33%). By stimulating with the IBA 500 and IBA 1000, the red currant has reached a very good rooting percentage, 90% of the cuttings have been rooted.

Observations and calculations were made on the type of the vegetative growths resulting from the evolution of the apical buds of the cutting. Thus, more shoots could be counted than leaf rosettes in the case of thicker cuttings; the thinner ones, even if they have recorded the best rooting percentage, have led to the formation of more rosettes of leaves (table 2).

The rooting hormones showed a more pronounced effect on the black currant related to the percentages of cutting's shoots, where the IBA 1500 and descending to the IBA 500 induced the occurrence of larger shoots. For red currant, the differences between the compounds used to stimulate the rooting of the cuttings were less evident; the control formed the highest number of shoots compared to the other cuttings.

Also, the highest number of rosettes was observed in the perlite + sand substrate (figure 4).

Table 2. Share of shoots and rosettes / currants cuttings (5 months from the begining)

Red currant	Substrate	Hormone/Product												Average	
		IBA 500		IBA 1000		IBA 1500		Razormin		Rhizopon		Control			
		S	R	S	R	S	R	S	R	S	R	S	R	S *	R*
		%	%	%	%	%	%	%	%	%	%	%	%	%	%
Thick cutting	Peat + Perlite	7	3	88.8	11.1	83.3	16.6			87.5	12.5	10		**85.94a**	**14.06a**
	Peat + Sand	7	3	83.3	16.6	66.6	33.3	5	5	83.3	16.6			**70.66a**	**29.34a**
	Perlite + Sand	77.7	22.2	9	1	7	3	10		77.7	22.2	10		**85.92a**	**14.08a**
	Average	***72.59***	***27.41***	***87.40***	***12.59a***	***73.33***	***26.66***	***75.00***	***25.00a***	***82.87***	***17.13a***	***100***	***0.00***	**80.84a**	**19.16a**
Thin cutting	Peat + Perlite	10		8	2	9	1	66.6	33.3	8	2	7	2	**81.94a**	**18.06b**
	Peat + Sand	7	2	7	3	5	5	9	1	57.1	42.8	66.6	33.3	**68.13ab**	**31.87b**
	Perlite + Sand	5	5	33.3	77.7	7	3	12.	87.	33.3	77.7	9	1	**44.49b**	**55.51a**
	Average	***75.00***	***25.00***	***57.41***	***42.59***	***70.00***	***30.00***	***56.39***	***43.61***	***53.12***	***46.88***	***77.22***	***22.78***	**64.86ab**	**35.14ab**
Overall mean		***73.80a***	***26.20a***	***72.41a***	***27.59a***	***71.67a***	***28.33a***	***65.69a***	***34.31a***	***67.99a***	***32.00a***	***88.61a***	***11.39a***	*72.85a*	*27.15a*
Black currant	Substrate	Hormone/Product												Average	
		IBA 500		IBA 1000		IBA 1500		Razormin		Rhizopon		Control			
		S	R	S	R	S	R	S	R	S	R	S	R	S *	R*
		%	%	%	%	%	%	%	%	%	%	%	%	%	%
Thick cutting	Peat + Perlite	75	25	75	25	90	10	62.5	37.5	83.33	16.66	50	50	**72.64b**	**27.36b**
	Peat + Sand	100	0	100	0	100	0	100	0	100	0	100	0	**100.00a**	**0.00c**
	Perlite + Sand	30	70	50	50	44.44	55.55	0	100	0	100	20	100	**24.07c**	**79.26a**
	Average	***68.33a***	***31.67a***	***75.00a***	***25.00a***	***78.15a***	***21.85a***	***54.17a***	***45.83a***	***61.11a***	***38.89a***	***56.67a***	***50.00a***	**65.57b**	**35.54b**
Thin cutting	Peat + Perlite	62.5	37.5	88.88	11.11	100	0	55.55	44.44	66.66	33.33	100	0	**78.93ab**	**21.06c**
	Peat + Sand	77.77	22.22	66.66	33.33	100	0	100	0	71.42	28.57	100	0	**85.98a**	**14.02c**
	Perlite + Sand	42.85	57.14	20	80	0	100	0	100	0	100	0	100	**10.48c**	**89.52a**
	Average	***61.04a***	***38.95a***	***58.51a***	***41.48a***	***66.67a***	***33.33a***	***51.85a***	***48.15a***	***46.03a***	***53.97a***	***66.67a***	***33.33a***	**58.46b**	**41.54b**
Overall mean		***64.69a***	***35.31a***	***66.76a***	***33.24a***	***72.41a***	***27.59a***	***53.01a***	***46.99a***	***53.57a***	***46.43a***	***61.67a***	***41.67a***	*62.02a*	*38.54a*

*S-shoot; R-rosette of leaves

*Duncan's multiple range test ($P \leq 0.05$)

Figure 4. The bigger share of rosettes formed by red currants cuttings in perlite + sand substrate (first two rows in the front) - 21.05.2016

The vigour of the rooted cuttings was highlighted by the length of the total growth of the cutting (figure 5).

The length of the shoots was influenced by all the experimental factors analysed and are presented in table 3 for each type of currant.

Regarding the type of cutting, we can say that this factor did not directly influence the length of the shoot/s, the differences being very small in the red currant and somewhat larger in the black currant (only 1.18 cm).

The same thing cannot be said about the type of substrate, which had a decisive role in plant height. Therefore, for the black currant, the most effective was the peat + sand substrate, which achieved an average of 16.79 cm. At the opposite side, there is the perlite with sand, the substrate which generated the lowest length of the shoot, and the biggest share of the rosettes/cutting. For the red currant, the best substrate proved to be peat + perlite.

Table 3. The length of the shoots/cutting (cm)

Red currant	Substrate	IBA 500	IBA 1000	IBA 1500	Razormin	Rhizopon	Control	Average
Thick cutting	Peat + Perlite	11.07	12.50	14.20	0.00	20.14	12.00	**11.65a**
	Peat + Sand	13.40	9.40	11.00	8.33	13.00	0.00	**9.19a**
	Perlite + Sand	8.57	7.88	5.67	5.50	7.00	6.25	**6.81a**
	Average	**11.01a**	**9.93a**	**10.29a**	**4.61a**	**13.38a**	**6.08a**	**9.22a**
Thin cutting	Peat + Perlite	13.14	10.50	12.89	8.00	11.25	11.33	**11.19a**
	Peat + Sand	10.50	9.07	11.25	11.22	10.63	11.08	**10.63ab**
	Perlite + Sand	7.20	4.00	4.57	6.00	6.00	5.67	**5.57c**
	Average	**10.28a**	**7.86a**	**9.57a**	**8.41a**	**9.29a**	**9.36a**	**9.13b**
Overall mean		**10.65ab**	**8.89ab**	**9.93ab**	**6.51b**	**11.34a**	**7.72ab**	**9.17ab**
Black currant	Substrate	IBA 500	IBA 1000	IBA 1500	Razormin	Rhizopon	Control	Average
Thick cutting	Peat + Perlite	7.17	8.91	8.05	4.8	12.4	2	**7.22b**
	Peat + Sand	17	22.17	19.83	11.75	17.16	15.75	**17.28a**
	Perlite + Sand	1.75	2.7	2.5	0	0	2	**1.49c**
	Average	**8.64a**	**11.26a**	**10.13a**	**5.52a**	**9.85a**	**6.58a**	**8.66ab**
Thin cutting	Peat + Perlite	12.2	13.31	12.8	12.8	15	7.05	**12.19b**
	Peat + Sand	16.71	18.67	22	14	13.2	13.22	**16.30a**
	Perlite + Sand	3.67	2.5	0	0	0	0	**1.03c**
	Average	**10.86a**	**11.49a**	**11.60a**	**8.93a**	**9.40a**	**6.76**	**9.84b**
Overall mean		**9.75ab**	**11.38a**	**10.86a**	**7.23bc**	**9.63abc**	**6.67c**	**9.25abc**

*Duncan's multiple range test (P≤0.05)

Figure 5. The stimulating effect of IBA hormone upon the length of the thicker black currants cuttings rooted in the sand + perlite substrate (7.06.2016)

To illustrate the quality of the cuttings, we proceed to assess the root volume of the black and red currant cuttings.

As can be seen from table 4, the black currant developed a bigger root system, which also explains the length of the shoots on same substrates.

In the red currant, both for the thicker and the thinner cuttings, the substrate that favoured the formation of a larger root volume was peat + sand (figure 6).

In the black currant, the differences were somewhat smaller, highlighting peat mixed with pearl or sand.

Table 4. The root volume of the currants cuttings (mm^3)

Red currant	Substrate	IBA 500	IBA 1000	IBA 1500	Razormin	Rhizopon	Control	Average
Thick cutting	Peat + Perlite	6.10	5.22	7.00	1.00	4.80	6.00	**5.02a**
	Peat + Sand	6.17	6.50	7.43	4.67	7.00	6.00	**6.29a**
	Perlite + Sand	3.89	4.80	3.40	7.00	4.67	3.75	**4.58a**
	Average	**5.39a**	**5.51a**	**5.94a**	**4.22a**	**5.49a**	**5.25a**	**5.30a**
Thin cutting	Peat + Perlite	4.29	2.50	3.56	2.67	3.90	4.20	**3.52bc**
	Peat + Sand	3.33	4.22	6.67	5.00	4.57	4.89	**4.78a**
	Perlite + Sand	3.14	2.60	2.60	2.44	2.60	2.80	**2.70c**
	Average	**3.59a**	**3.11a**	**4.27a**	**3.37a**	**3.69a**	**3.96a**	**3.67b**
Overall mean		**4.49a**	**4.31a**	**5.11a**	**3.80a**	**4.59a**	**4.61a**	**4.48a**
Black currant	Substrate	IBA 500	IBA 1000	IBA 1500	Razormin	Rhizopon	Control	Average
Thick cutting	Peat + Perlite	2.60	3.11	2.00	1.75	3.71	2.50	**2.61abc**
	Peat + Sand	2.80	4.33	3.25	3.50	2.25	2.57	**3.12a**
	Perlite + Sand	2.20	2.00	2.22	2.80	2.88	2.10	**2.37bc**
	Average	**2.53a**	**3.15a**	**2.49a**	**2.68a**	**2.95a**	**2.39a**	**2.70ab**
Thin cutting	Peat + Perlite	2.57	2.67	2.60	2.22	2.22	2.40	**2.45a**
	Peat + Sand	1.60	2.25	2.50	2.40	2.25	2.50	**2.25a**
	Perlite + Sand	2.22	1.80	1.33	1.56	1.67	0.80	**1.56c**
	Average	**2.13a**	**2.24a**	**2.14a**	**2.06a**	**2.05a**	**1.90a**	**2.09ac**
Overall mean		**2.33a**	**2.69a**	**2.32a**	**2.37a**	**2.50a**	**2.15a**	**2.39a**

*Duncan's multiple range test (P≤0.05)

IBA 1000 and IBA 1500 presented the highest efficiency in providing a broader radicular system, managing the highest values in both species of currant. But the differences were not significantly evident from the other variants.

Figure 6. Detail of the root system generated by red currants thick cuttings stimulated with IBA 1000 ppm in peat + sand substrate (left) versus perlite + sand (right)

Regardless of the variants analysed, we can emphasize the clear difference between the ability to form more roots / cutting in the case of the black currant compared to the red currant. Thus, about 65% of the total roots belong to the black currant cuttings while only 35% of the red currant.

CONCLUSIONS

The rooting percentage of cuttings was of 77.22% for the black currant variety 'Tinker' and 77.36% for the 'Elite' red currant variety.
For both varieties, the thinner cuttings, with a diameter between 0.3-0.48 mm (black currant) and 0.26-0.35 mm (red currant) gave the best results in terms of rooted cuttings percentage.
The perlite + sand substrate achieved the highest rooting percentage of the cuttings but with the lowest vegetative growths, also with higher share of rosettes vs shoots.
Among the stimulators used for rooting, the IBA hormone (regardless of the concentration used) achieved the best percentages of cuttings rooted in both currant varieties.
The mixture of perlite + peat for 'Tinker' variety, has contributed to the appearance of a larger number of shoots than the other tested substrates.
For the black currant, the sandy peat substrate favoured the development of the most vigorous shoots, its average length being 16.79 cm with a maximum of 22.17 cm.
The IBA 1000 ppm gave the best results on the length of the shoots on both currant varieties.
The root volume in the red currant was positively influenced by the peat + sand mixture regardless to the thickness of the cuttings.
For black currant, better results of root volume were recorded when the perlite + peat mixture was used as substrate for rooting.
The volume of the root system in the red currant was approximately 2 times larger than the black currant.
IBA 1000 and IBA 1500 gave the most extensive radicular system to both currant varieties.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, project number PN-II-RU-TE-2014-4-0749.

REFERENCES

Caulet R.P., Onofrei O., Morariu A., Iurea D., Gradinaru G., 2012. Effect of furostanol glycoside treatments in plant material production in currants (*Ribes* sp.), Lucrari Stiintifice–Seria Horticultura, Ed. "Ion Ionescu de la Brad", Iasi, 54 (2): 231- 237.

De Klerk G.J., Van der Krieken W., De Jong J., 1999. Review the formation of adventitious roots: new concepts, new possibilities. *In Vitro* Cellular & Developmental Biology - Plant, 35(3): 189-199.

Gehlot A., Gupta R. K., Tripathi A., Arya I. D., 2014. Vegetative propagation of *Azadirachta indica*: effect of auxin and rooting media on adventitious root induction in mini-cuttings. Adv. For. Sci., 1(1): 1-9.

Hartmann H.T., Kester D.E., Jr. Davies F.T., 1990. Plant Propagation: Principles and Practices. 5th Edn., Prentice-Hall Inc., Englewood, Cliffs, New Jersey, USA.

Khudhur S.A., Omer T.J., 2015. Effect of NAA and IAA on Stem Cuttings of Dalbergia Sissoo (Roxb). Journal of Biology and Life Science, 6(2): 208-220

Koyuncu F., Şenel E., 2003. Rooting of Black Mulberry (*Morus nigra* L.) Hardwood Cuttings. Journal of Fruit and Ornamental Plant Research, 11: 53-57.

Kroin J., 2016. Hortus Plant Propagation from Cuttings. A guide to using plant rooting hormones by foliar and basal methods. Third Edition. Hortus USA Corp, PO Box 1956, New York NY 10113. support@hortus.com

Pandey A., Tamta S., Giri D., 2011. Role of auxin on adventitious root formation and subsequent growth of cutting raised plantlets of *Ginkgo biloba* L. International Journal of Biodiversity and Conservation, 3(4): 142-146.

Pop T.I., Pamfil D., 2011. Auxin Control in the Formation of Adventitious Roots. Not Bot Hort Agrobot Cluj, 39 (1): 307-316

Sandor F., 2007. Vegetative propagation techniques. Perrenial crop support series. Jalalabad, Afganistan, 2007-003-AFG, Roof of Peace, 13-15.

Siksnianas T., Sasnauskas A., Šterne D., 2006. The propagation of currants and gooseberries by softwood and combined cuttings. Agronomijas Vēstis 9: 135-139.

Szecsko V., Csikos A., Hrotko K., 2002. Timing of hardwood cuttings in the propagation of plum rootstocks. Acta Hortic. 577: 115-119

Szecsko V., Csikos A., Hrotko K., 2002. Timing of hardwood cuttings in the propagation of plum rootstocks. Acta Hortic. 577: 115-119.

Uniyal R.C., Prasad P., Nautiyal A.R., 1993. Vegetative propagation in *Dalbergia sericea*: Influence of growth hormones on rooting behaviour of stem cuttings. J. Trop. For. Sci., 6: 21-25.

Wiesman Z., Riov J., Epstein E., 1988. Comparison of movement and metabolism of indole-3-acetic acid and indole-3-butyric acid in mung bean cuttings. Physiologia Plantarum, 74(3): 556-560.

EX·TERRA·AVRVM

VITICULTURE
AND OENOLOGY

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECTS OF ADDING GLUTATHIONE AND ASCORBIC ACID BEFORE THE ALCOHOLIC FERMENTATION OF THE MUSTS ON THE SENSORY PROFILE OF THE WHITE WINES

Gianina Antonela BADEA, Valerica TUDOR, Răzvan Ionuț TEODORESCU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59, Mărăşti Blvd., District 1, Bucharest, Romania

Corresponding author email: razvan.teodorescu@usamv.ro

Abstract

Considering the fact that in the last decade consumers' preference turned to fresh white wines with a strong aromatic profile, wine producers have set as a priority the preservation of varietal aromas from the early stages of primary winemaking during all the process of wine production to the final product, and then, after bottling, until final consumption. In this direction, new antioxidants have been proposed by experts, and the natural antioxidant glutathione (GSH), on which winemakers have great expectations, was recently approved for use in must and wine by OIV.
The present work evaluates the influence of using different doses of reduced glutathione (GSH), as such or in combination with ascorbic acid (AA), on the sensory profile of produced wines, treatments applied before the inoculation with selected yeasts of the musts produced in reductive manner in the presence of sulphur dioxide (SO_2), from Sauvignon Blanc and Muscat Ottonel grapes.The results of the study show that the type and dosage of antioxidants have a significant influence on the sensory profiles of obtained wines and that a higher dosage than 20 mg/L approved by the OIV could be necessary to obtain wines with sensory profiles more appreciated by the consumers.

Key words*: white wine aromas, sensory profile, glutathione, ascorbic acid, Sauvignon Blanc, Muscat Ottonel.*

INTRODUCTION

Oxidation is the main cause for the loss of musts' and wines' varietal aromatic characteristics (Kilmartin, 2010). Therefore, choosing the way of antioxidant protection during winemaking, from the initial crushing of the grapes and till the alcoholic fermentation beginning, is extremely important for defining the style and maintaining the quality of the wine we wish to produce.

Without an adequate management of the must contact with oxygen oxidation reactions of phenolic compounds that give wine and typical varietal character can occur. Oxidative changes start since the time of the grape harvest and during the transport to the cellar due to the contact with the oxygen of the must originating from broken berries (Badea and Antoce, 2015). Under the action of absorbed oxygen, phenolic compounds oxidation in musts takes place simultaneously both by chemical (non-enzymatic) and enzymatic mechanisms (Antoce, 2007; Badea and Antoce, 2015). Simultaneously with destemming and crushing the grapes, in contact with the oxygen and in the presence of polyphenoloxidases (PPOs) derived from healthy grapes such as tyrosinase, cresolase, and catechol oxidase and of laccase enzymes associated with *Botrytis cinerea* fungus contaminated grapes, the oxidation process of the phenolic compounds responsible for the wine aromas and of the flavour precursors starts (du Toit and Oberholster, 2014; Badea and Antoce, 2015). The enzymatic oxidation affects mostly the cinnamic acid and its esters with tartaric acid (caftaric acid and cutaric acid) causing the formation of quinones, easy oxidable compounds further participating to reactions of oxidation and oxidative polymerization (du Toit and Oberholster, 2014; Badea and Antoce, 2015). The quinones formed from the caffeic acid oxidation have a high affinity to react with catechins resulting catechin quinones, which further participate to condensation reaction (Cheynier et al., 1989). The enzymatic oxidation of the phenolic compounds occurs mostly during the berries' crushing operation, but other non-enzymatic oxidation reactions can occur later (Sonni et al., 2011 a; Ugliano et al., 2011). Non-enzymatic oxidation can also occur in musts leading to similar chemical processes and the formation of same undesirable compounds through the action of hydrogen peroxide formed from the oxidation of ortho-catechins to ortho-diquinones (Singleton, 1987; Antoce, 2007). The processes continue throughout all the applied technological operations until the start of alcoholic fermentation of the must (du Toit et al., 2006).

This is why antioxidant agents such as sulphur dioxide (SO_2) and ascorbic acid (AA), whose action is to reduce or eliminate quinones, are used in the winemaking for the control of the oxidative processes which take place in musts and wines and to assure a better protection of varietal aromas and stability of wines (Brajkovich et al., 2005; Lavigne Cruège et al., 2003; Ugliano et al., 2011). The sulphur dioxide is used in winemaking as it has both capacities to protect musts from enzymatic oxidation and antimicrobial properties (Garde-Cerdán and Ancín-Azpilicueta, 2007). The ascorbic acid is used in different combinations and doses together with sulphur dioxide because of their abilities to inhibit the musts' and wines' polyphenol oxidation (Oliveira et al., 2002). However, because it is well known the allergenic potential of sulphur dioxide and the belief that its use represents a risk to human (Walker, 1985; Garde-Cerdán and Ancín-Azpilicueta, 2007) and researchers have shown that ascorbic acid addition can lead to sotolon formation and can damage the wine aromas (Pons et al., 2010) other solutions must be found for the antioxidant protection of musts and wines. In the last years, the reduced glutathione (GSH), an antioxidant naturally found in grapes was the subject of research for its capacity to protect varietal aromas of musts or wines. Quantities of GSH from small traces to 100 mg/L or more, depending on the variety, oxygen absorption, enzymatic activity and the applied technology (crushing, maceration and pressing conditions) have been measured in grape musts (Cheynier et al., 1989; Park et al., 2000; du Toit et al., 2007; Maggu et al., 2007; Patel et al., 2010), but at least 50-100 mg/L of free GSH in the crushed grapes are necessary for musts antioxidant protection (Singleton et al., 1985). It was already shown that GSH has the capacity to react with caftaric acid quinones leading to 2-S-glutathionyl caftaric acid, also named Grape Reaction Product (GRP) and protect the musts against the browning phenomenon (Singleton et al., 1985; Cheynier et al., 1986; Du Toit et al., 2006; Antoce, 2007; Sonni et al., 2011 a, b). Vaimakis and Roussis (1996) reported that the addition of GSH to oxygenated musts determined the achievement of wines free of oxidation typical aromas and with a level of quality obviously improved. Du Toit and his collaborators (2006) have shown that the treatment with GSH confer to wines a better aromatic profile. Webber and his team (2014) have shown that the addition of 10-20 mg/L GSH to the base must and not in the base wine, led to better quality sparkling wines.

Therefore in July 2015 the addition of up to 20 mg/L GSH to must and wine became an approved oenological practice by OIV (Resolutions OENO-TECHNO 10-445 and 10-446 / July 2015).

In the present work the sensory profiles of two wines, Sauvignon Blanc and Muscat Ottonel have been studied, aiming to evaluate the effect of different doses of glutathione addition as such or in combination with ascorbic acid in sulphited musts, added in clarified musts prior to the alcoholic fermentation initialisation.

MATERIALS AND METHODS

The wine samples have been prepared from Sauvignon Blanc and Muscat Ottonel respectively clarified musts with a certain free SO_2 content. The musts have been produced in a reductive manner by treating the grapes in the receiving tank with 1 g/kg potassium metabisulphite and very small quantities of catechin tannin (0.045 - 0.05 g/kg).

A very small quantity of AA of 0.13 g/kg has been administered on Sauvignon Blanc grapes in the receiving tank. Various dosages of GSH as such or in combination with ascorbic acid have been applied in both types of musts.

The base must of Sauvignon Blanc, with a sugar content of 212 g/L, was produced between the 10^{th} and 12^{th} of September 2015 in the Domeniile Dealu Mare Urlati winery, Prahova County, Romania, from grapes originating from an eight year old plantation (2007).

The base must of Muscat Ottonel, with a sugar content of 210 g/L, was produced between the 25^{th} and the 26^{th} of September 2015 in the same winery, from grapes originating from a more than 40 years old vineyard.

The technology used for producing both types of musts was the same. After destemming and crushing the grapes, followed by maceration on the skins performed directly in the press for 12 hours, the must drained by gravity without pressing the grapes was subjected to clarification by refrigeration at 10°C in the presence of pectolytic enzymes in tanks with cooling jacket. Musts acidity corrections with tartaric acid (1.4 - 1.65 g/L) and SO_2 correction up to min. 50 mg/L were performed.

The musts produced in this manner were treated in 25 L demijohns with different doses of reduced glutathione (GSH), as such or in combination with a dose of 50 mg/L ascorbic acid (AA).

The experimental variants and the type of treatments are presented in Table 1.

Table 1. Experimental research variants

Experimental variant	Type of Treatment
Musts from Sauvignon Blanc and Muscat Ottonel grape varieties produced in September 2015 treated prior to inoculation with selected yeasts (free SO_2 content: min. 50 mg/L)	GSH doses of 20 mg/L or 40 mg/L
	GSH doses of 20 mg/L or 40 mg/L and AA fixed dose of 50 mg/L

The treatments were applied to musts prior to the inoculation with selected yeast and activators used to initiate the controlled alcoholic fermentation.

The wine samples were prepared in 5 variants and 3 repetitions of each version for both types of musts (Table 2).

Table 2. Variants of musts and administered treatments

Grape variety	Must Sample Code	Dosage
Sauvignon Blanc	SBControl	Control, without treatment
	SBGSH20AA00	20 mg/L Glutathione and 0 mg/L Ascorbic Acid
	SBGSH20AA50	20 mg/L Glutathione and 50 mg/L Ascorbic Acid
	SBGSH40AA00	40 mg/L Glutathione and 0 mg/L Ascorbic Acid
	SBGSH40AA50	40 mg/L Glutathione and 50 mg/L Ascorbic Acid
Muscat Ottonel	MOControl	Control, without treatment
	MOGSH20AA00	20 mg/L Glutathione and 0 mg/L Ascorbic Acid
	MOGSH20AA50	20 mg/L Glutathione and 50 mg/L Ascorbic Acid
	MOGSH40AA00	40 mg/L Glutathione and 0 mg/L Ascorbic Acid
	MOGSH40AA50	40 mg/L Glutathione and 50 mg/L Ascorbic Acid

The same winemaking protocols were applied to both types of musts, as it can be seen in Figure 1.

Primary Vinification Protocol
Sauvignon Blanc / Muscat Ottonel

↓

In the clarified musts: SO_2 correction up to 50 mg/L and then the established doses of GSH (20 mg/L or 40 mg/L GSH) and AA (50 mg/L or 0 mg/L) are administered

↓

The addition of the first dose of activator - 300 mg/L V Starter Premium

↓

Selected Yeast Inoculation: 20 mg/L (Premium Sauvignon Blanc/ Premium Blanc) and fermentation at 15 – 16 °C

↓

The second dose of activator addition during the tumultuous alcoholic fermentation phase - 200 mg/L V Activ

↓

At the end of the alcoholic fermentation (sugar < 5 g/L) - addition of PVPP (0.1 - 0.15 g/L), the wine is pulled from the yeast deposit, SO_2 correction up to 20 mg/L free SO_2 and addition of 15 mg/L Mannozym

↓

Conservation for a month with weekly batonnage of the fine yeast deposit (free SO_2 maintained at min. 20 mg/L)

Figure 1. Primary vinification protocols applied to the studied musts

All the oenological materials were supplied by Enologica Vason Italy.

The obtained wine samples were analysed freshly, immediately after the end of the winemaking and before bottling.

The sensory evaluation of the obtained wines was performed directly into the cellar by a group of three authorised wine tasters based on two evaluation sheets: the unified card OIV UIOE used for international competitions - for still wines, and an evaluation tasting form specially designed (Stoian, 2011; Antoce and Namolosanu, 2007).

On the unified card OIV UIOE (Figure 2), the wines were evaluated by providing scores for the visual aspect (limpidity, aspect), in terms of odour (genuineness, intensity, quality), taste (genuineness, intensity, persistence, quality) and overall harmony.

Fiche unifieé OIV UIOE á l'usage des concours internationaux: VINS TRANQUILLES
Unified card OIV UIOE used for international wine contests: STILL WINES
Fişă comună OIV UIOE pentru utilizare la concursuri internţionale: VINURI LINIŞTITE

Commission n°./ Commission no./ Comisia nr.	Nom du dégustateur/ Judge name/ Numele degustătorului	Sample/ Échantillon/ Proba

		Excellent Excellent Excelent	Trés bon Very Good Foarte bun	Bon Good Bun	Satisfaisant Satisfactory Satisfăcător	Insuffisant Insufficient Insuficient	Remarques Observations Observații
VUE/ VISUAL/ VIZUAL	Limpidité/ Limpidity/ Limpiditate	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1	
	Aspect/ Aspect/ Aspect	☐ 10	☐ 8	☐ 6	☐ 4	☐ 2	
ODORAT/ SMELL/ MIROS	Franchise/ Genuineness/ Tipicitate	☐ 6	☐ 5	☐ 4	☐ 3	☐ 2	
	Intensité/ Intensity/ Intensitate	☐ 8	☐ 7	☐ 6	☐ 4	☐ 2	
	Qualité/ Quality/ Calitate	☐ 16	☐ 14	☐ 12	☐ 10	☐ 8	
GOÛT/ TASTE/ GUST	Franchise/ Genuineness/ Tipicitate	☐ 6	☐ 5	☐ 4	☐ 3	☐ 2	
	Intensité/ Intensity/ Intensitate	☐ 8	☐ 7	☐ 6	☐ 4	☐ 2	
	Persistance/ Persistence/ Persistență	☐ 8	☐ 7	☐ 6	☐ 5	☐ 4	
	Qualité/ Quality/ Calitate	☐ 22	☐ 19	☐ 16	☐ 13	☐ 10	
HARMONIE / GLOBAL JUGEMENT HARMONY / OVERALL JUDGEMENT ARMONIE / EVALUARE GLOBALĂ		☐ 11	☐ 10	☐ 9	☐ 8	☐ 7	
						TOTAL	

Signature du dégustateur/ Judge signature/ Semnătura degustătorului	
Signature du président/ President signature/ Semnătura președintelui	

Figure 2. Model of unified card OIV UIOE for evaluation of still wines used in international competitions (reproduced after Stoian, 2011)

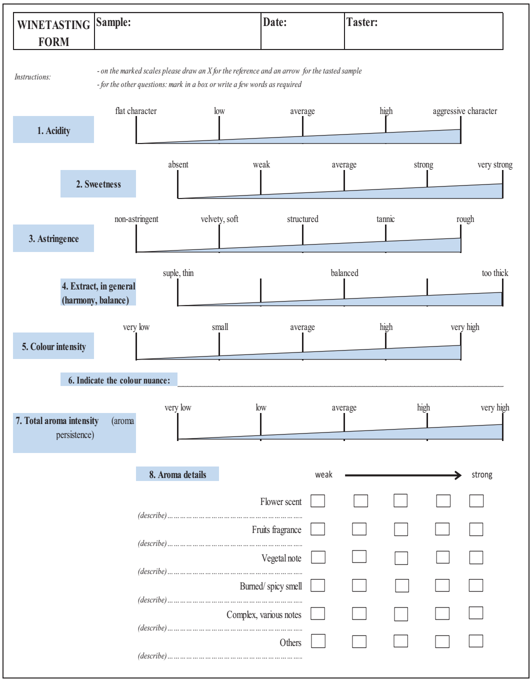


Figure 3. Model of wine tasting form for evaluation of still wines (reproduced after Antoce and Namolosanu, 2007)

On the second tasting form, the wines were first evaluated according to some basics characteristics of the wines, like the acidity, the sweet taste, the extract (Figure 3).

The evaluation methodology is based on scales of 10 cm length, which can be very easy converted in values from 0 to 10 by direct measurement with a normal ruler on the data sheet, scale on which the tasters indicate the level evaluated for each of the analysed characteristics.

These scales have a specific triangle shape, with the sharpest angle pointing towards the left and the base pointing to the right, suggesting that values are increasing in this direction (Antoce, 2007).

Furthermore, some anchors are marked corresponding to each evaluation criteria on the largest triangle leg, as follows:

- acidity: flat character, low, average, high, aggressive character;
- •sweetness: absent, weak, average, strong, very strong;
- •astringency: non-astringent, velvety / soft, structured, tannic, rough;
- •extract, in general (harmony, balance): supple / thin, balanced, too thick;
- •colour intensity: very low, small, average, high, very high.

As regarding the specific parameters of the evaluated wines, the hue and the flavour intensity, the wine tasters had to directly describe on the evaluation form the colour nuance of the wines they have observed.

They also had to evaluate the overall flavour intensity of the wines by indicating the level they have observed on the scale from 1 to 10 with some anchors (very low, low, average, high, very high), available on the tasting sheet for the aroma persistence (Antoce, 2007).

The last evaluation the wine tasters had to achieve is the description of flavour specifying the aroma details by ticking one of the five squares strung from left to right on the evaluation form, rating six flavour nuances from weak to strong: flower scent, fruits fragrance, vegetal note, burned / spicy smell, complex, various notes and other nuances they identified. Other comments can be mentioned on the evaluation form if there are special observations regarding the aroma details.

Because the evaluators did not noticed big sensory differences between the repetitions of the same treatment variant, average samples were made by combining the 3 repetitions for each experimental variant and for both wines Sauvignon Blanc and Muscat Ottonel.

These average wine samples have been then evaluated according the wine sensory evaluation forms previously described and scores have been awarded for each type of wine sample.

The numerical results determined by measuring the assessment of each taster have been grouped into tables and the average scores for each parameter and each wine sample have been calculated.

The main observations made by wine tasters on aroma details have been also centralized in tables for each wine variety and samples variants. Based on the obtained results, the sensory profile of each evaluated wine sample was built for both varieties of wine. The sensory profiles have been reported as a spider web chart and a description of the main characteristics of the analysed samples.

Also, based on the obtained scores on the unified card OIV UIOE, the ranking of analysed wine samples was achieved and the most appreciated wine sample submitted to the tasters' sensory evaluation from each variety of wine was identified.

As a result, the treatments applied to the samples of musts from which the most appreciated wines are originating and the doses of antioxidants added prior the initialization of the alcoholic fermentation of samples have been determined.

RESULTS AND DISCUSSIONS

Physic-chemical parameters of the Sauvignon Blanc wine samples produced with experimental protocols proposed for research that have correspondent with the wines characteristics evaluated on the sensory analysis sheets are listed in Table 3.

The Muscat Ottonel wine samples' physic-chemical parameters that have correspondence with the wines characteristics evaluated on the sensory analysis sheets are presented in Table 4.

Table 3. Physico-chemical parameters of the Sauvignon Blanc wines determined before bottling

Wine sample code	Total acidity (g/L tartaric ac.)	Sugar (g/L)	Nonreducing dry extract (g/L)
SBControl			
SBGSH00AA00 R1	7.8	2.6	22.4
SBGSH00AA00 R2	8.1	3.1	22.9
SBGSH00AA00 R3	8.2	7.8	18
SBGSH20AA00			
SBGSH20AA00 R1	7.6	2.7	22
SBGSH20AA00 R2	6.84	2.3	18
SBGSH20AA00 R3	6.31	2.37	19.53
SBGSH20AA50			
SBGSH20AA50 R1	6.31	2.37	20.83
SBGSH20AA50 R2	7.74	2.6	22.7
SBGSH20AA50 R3	6.39	4.1	19.5
SBGSH40AA00			
SBGSH40AA00 R1	6.39	2.62	21.48
SBGSH40AA00 R2	6.4	3.18	22.02
SBGSH40AA00 R3	6.54	1.6	18.1
SBGSH40AA50			
SBGSH40AA50 R1	6.31	1.81	20.19
SBGSH40AA50 R2	6.3	3.56	19.2
SBGSH40AA50 R3	6.24	2.37	19.83

Table 4. Physico-chemical parameters of the Muscat Ottonel wines determined before bottling

Wine sample code	Total acidity (g/L tartaric ac.)	Sugar (g/L)	Nonreducing dry extract (g/L)
MOControl			
MOGSH00AA00 R1	8.4	6.5	21.6
MOGSH00AA00 R2	7.74	8.1	17.6
MOGSH00AA00 R3	8.1	7.5	20
MOGSH20AA00			
MOGSH20AA00 R1	8	7.6	19.5
MOGSH20AA00 R2	7.9	7.12	19.78
MOGSH20AA00 R3	7.9	5.87	20.83
MOGSH20AA50			
MOGSH20AA50 R1	7.74	5.87	20.1
MOGSH20AA50 R2	7.8	5.43	20.67
MOGSH20AA50 R3	8.5	6.5	19.7
MOGSH40AA00			
MOGSH40AA00 R1	8.4	6.87	20.33
MOGSH40AA00 R2	8.9	5.8	23.4
MOGSH40AA00 R3	8.7	8.31	20.39
MOGSH40AA50			
MOGSH40AA50 R1	8.7	6.25	19.85
MOGSH40AA50 R2	8	2.6	25
MOGSH40AA50 R3	7.2	5.87	20.63

The scores awarded by tasting jurors on the OIV UIOE unified card and the average marks calculated for the Sauvignon Blanc wine samples are shown in Table 5.

Table 5. The scores on the OIV UIOE unified card and the calculated average scores for Sauvignon Blanc wines

Wine sample Code/ Judge Name	Visual		Odour			Taste				Harmony/ Overall judgement	Total
	Limpidity	Aspect	Genuiness	Intensity	Quality	Genuiness	Intensity	Persistence	Quality		
SBControl	**3.77**	**6.35**	**4.41**	**6.41**	**12.64**	**5.00**	**6.91**	**7.00**	**17.22**	**10.00**	**79.70**
Judge 1	4	7	5	7	13	5	7	7	18	10	81
Judge 2	4	6	5	7	13	5	7	7	18	10	81
Judge 3	4	6	4	6	12	5	7	7	16	10	77
SBGSH20AA00	**3.67**	**7.33**	**5.00**	**7.00**	**13.33**	**5.00**	**6.67**	**7.00**	**19.00**	**10.00**	**84.00**
Judge 1	3	6	5	7	14	5	7	7	19	10	83
Judge 2	4	8	5	7	14	5	7	7	19	10	86
Judge 3	4	8	5	7	12	5	6	7	19	10	83
SBGSH20AA50	**3.33**	**6.00**	**5.00**	**6.33**	**13.33**	**5.00**	**7.00**	**7.00**	**17.00**	**10.00**	**80.00**
Judge 1	3	6	5	6	14	5	7	7	19	10	82
Judge 2	3	6	5	6	12	5	7	7	16	10	77
Judge 3	4	6	5	7	14	5	7	7	16	10	81
SBGSH40AA00	**3.67**	**8.00**	**5.00**	**6.33**	**14.00**	**5.00**	**7.00**	**7.67**	**19.00**	**10.00**	**85.67**
Judge 1	4	8	5	7	14	5	7	7	19	10	86
Judge 2	3	8	5	6	14	5	7	8	19	10	85
Judge 3	4	8	5	6	14	5	7	8	19	10	86
SBGSH40AA50	**3.00**	**6.00**	**4.33**	**6.00**	**12.00**	**4.33**	**6.33**	**6.67**	**16.00**	**9.00**	**73.67**
Judge 1	3	6	5	6	12	4	6	7	16	9	74
Judge 2	3	6	4	6	12	4	6	7	16	9	73
Judge 3	3	6	4	6	12	5	7	6	16	9	74

After the UIOE regulations awards are granted to wines receiving the following scores: over 90 points - Gold Medal, between 85 and 90 points - Silver Medal, between 80 and 85 points - Bronze Medal and between 75 and 80 points – Certificate of merit (Stoian, 2011).

The grille used in the last years at the international wine contests organized in Romania under the supervision OIV for awarding the wines which will be valid also for the 2017 contest is a bit harsher than the UIOE grille: over 92 points - Great Gold Medal, between 85 and 91.99 points - Gold Medal, between 82 and 84.99 points - Silver Medal (IWCB 2017 Regulations).

By analyzing the average scores for the Sauvignon Blanc wines (Table 5) we can observe that scores between 73.67 points and 85.67 points have been resulted. If we consider the grille used according the ICWB regulations, then both wine samples treated only with GSH could reach awards, the sample SBGSH40AA00 with 85.67 points – Gold Medal and the sample SBGSH20AA00 with 84.00 points – Silver Medal. The control sample SBControl without any treatment and the samples treated with GSH and AA have been evaluated with lower grades (80.00 points, 73.67 points respectively) and would not have been qualified for any distinction.

From the graphic representation of the total average scores of Sauvignon Blanc wine samples evaluated freshly before bottling (Figure 4) it is easy to remark that wine samples produced only with GSH received higher scores, even if compared with the control sample SBControl without treatment or to wine samples produced with GSH and AA.

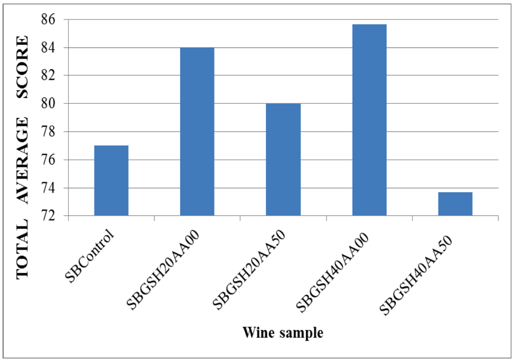


Figure 4. Graphic representation of the total average scores calculated for Sauvignon Blanc wines

The wine sample SBGSH40AA00 produced with a dose of 40 mg/L GSH and without AA was considered the best by all the judges. AA caused a deterioration of the wines' sensory profile and samples thus treated were downgraded properly by the wine tasters. This deterioration is more obvious if the dose of GSH increases.

If we follow the same reasoning for the Muscat Ottonel samples scores' (Table 6), we can conclude that the samples containing GSH, both with or without AA, obtained higher grades (from 82.67 to 86.67 points) than the control sample (79 points).

The samples treated only with GSH, MOGSH40AA00 and MOGSH20AA00 could qualify for the gold medal and silver medal (86.67 and 84.33 points respectively). Muscat Ottonel samples treated with GSH and the fixed dose of 50 mg/L AA were less downgraded by judges (82.67 and 83.33 points respectively) than the similar treated Sauvignon Blanc samples (80.00 and 73.67 points respectively).

In this case the sample MOGSH20AA50 was scored similarly with MOGSH20AA00 and would qualify for the silver medal.

Table 6. The scores on the OIV UIOE unified card and the calculated average scores for Muscat Ottonel wines

Wine sample Code/ Judge Name	Visual		Odour			Taste				Harmony/ Overall judgement	Total
	Limpidity	Aspect	Genuiness	Intensity	Quality	Genuiness	Intensity	Persistence	Quality		
MOControl	**4.00**	**7.00**	**5.00**	**6.50**	**13.00**	**4.50**	**6.50**	**6.50**	**16.00**	**9.00**	**78.00**
Judge 1	3	6	5	6	12	4	7	7	16	9	75
Judge 2	5	8	5	7	14	5	6	6	16	9	81
Judge 3	5	8	5	7	12	5	7	6	16	10	81
MOGSH20AA00	**4.50**	**8.00**	**5.00**	**6.50**	**13.00**	**5.00**	**6.50**	**6.50**	**19.00**	**9.50**	**83.50**
Judge 1	4	8	5	7	14	5	7	7	19	10	86
Judge 2	5	8	5	6	12	5	6	6	19	9	81
Judge 3	4	8	5	7	14	5	7	7	19	10	86
MOGSH20AA50	**4.50**	**9.00**	**5.00**	**7.00**	**13.00**	**5.00**	**6.00**	**7.00**	**17.50**	**9.50**	**83.50**
Judge 1	4	8	5	7	14	5	6	7	19	10	85
Judge 2	5	10	5	7	12	5	6	7	16	9	82
Judge 3	4	8	5	6	12	5	6	6	19	10	81
MOGSH40AA00	**4.00**	**9.00**	**5.00**	**7.00**	**14.00**	**5.00**	**7.00**	**6.50**	**19.00**	**10.00**	**86.50**
Judge 1	4	8	5	7	14	5	7	7	19	10	86
Judge 2	4	10	5	7	14	5	7	6	19	10	87
Judge 3	4	10	5	7	14	5	7	6	19	10	87
MOGSH40AA50	**4.00**	**8.00**	**5.00**	**6.50**	**14.00**	**5.00**	**6.50**	**6.50**	**17.50**	**10.00**	**83.00**
Judge 1	4	8	5	7	14	5	7	7	16	10	83
Judge 2	4	8	5	6	14	5	6	6	19	10	83
Judge 3	5	10	5	6	14	5	7	6	16	10	84

The control sample MOControl and the sample MOGSH40AA50 treated with 40 mg/L GSH and 50 mg/L were less appreciated by the wine tasters and would not have been qualified for any distinction.

From the graphic representation of the total average scores of Muscat Ottonel wine samples

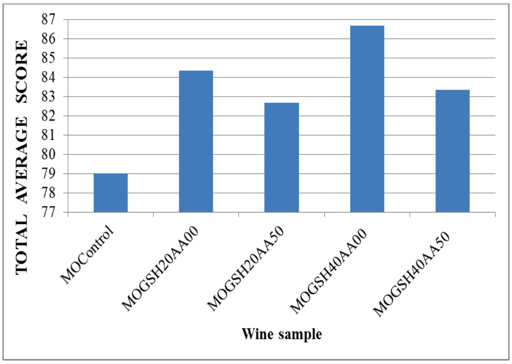


Figure 5. Graphic representation of the total average scores calculated for Muscat Ottonel wines

evaluated freshly before bottling (Figure 5) we can immediately notice that in this case the sample treated with 40 mg/L GSH and without AA obtained the highest score and was most appreciated by the jury as for the Sauvignon Blanc wine samples.

Average scores of the numerical values determined from the measurement of each tasters' marks have been calculated the for each parameter and each wine sample (Table 7 and Table 8).

Table 7. The calculated average scores on the second evaluation form for Sauvignon Blanc wines

Wine characteristics	SBControl	SBGSH20AA00	SBGSH20AA50	SBGSH40AA00	SBGSH40AA50
Acidity	4.3	4.7	4.7	4.2	4.3
Sweetness	0.5	0.8	0.3	0.5	1.2
Astringence	4.0	3.0	4.5	3.8	4.7
Extract	4.7	4.3	4.7	4.0	4.5
Colour intensity	4.3	4.5	4.8	4.7	6.2
Aroma persistence	2.8	4	4.3	5.5	3.3

Table 8. The calculated average scores on the second evaluation form for Muscat Ottonel wines

Wine characteristics	MOControl	MOGSH20AA00	MOGSH20AA50	MOGSH40AA00	MOGSH40AA50
Acidity	4.7	4.5	4.5	4.7	4.3
Sweetness	0.3	0.5	1.0	0.5	1.0
Astringence	4.0	3.3	3.7	3.7	4.5
Extract	4.2	4.0	4.0	3.7	4.3
Colour intensity	4.5	4.7	4.5	4.3	4.3
Aroma persistence	4.8	4.3	4.3	5.0	4.2

Based on these numerical values the sensory profile of each evaluated sample was built for both varieties of wine and represented as a spider web chart (Figure 6 and Figure7).

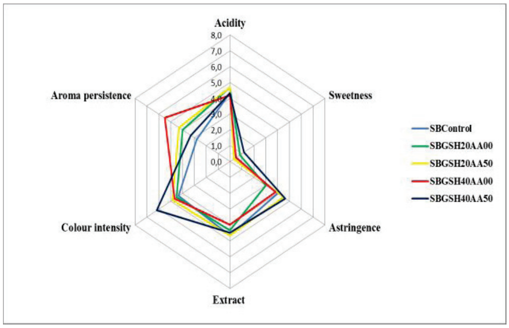


Figure 6. Graphic representation of the sensory profile determined for Sauvignon Blanc wine samples

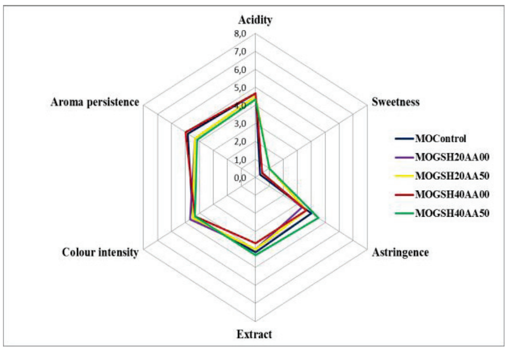


Figure 7. Graphic representation of sensory profile determined for Muscat Ottonel wine samples

By analyzing the sensory profile of Sauvignon Blanc wine samples of resulting from sensory analysis performed by experts we can see that all wines originating from Sauvignon Blanc musts treated with GSH, with or without AA, have a level of persistence of flavor and color intensity higher than the tasters reported for the control sample. The tasters have assessed wines produced from musts treated with GSH as balanced, structured and with a more complex sensory profile. AA presence determined an increase of the astringency and the intensification of bitter note, both compared to the control sample and samples produced only with GSH, the last ones being characterized as soft and velvety. The SBGSH40AA00 sample treated with 40 mg/L GSH has been evaluated as the sample with the best aroma persistence level and with the most balanced characteristics compared with all other Sauvignon Blanc samples (Table 9).

Table 9. Wine characteristics evaluation on the second tasting form for Sauvignon Blanc wines

Wine characteristics	SBControl	SBGSH20AA00	SBGSH20AA50	SBGSH40AA00	SBGSH40AA50
Acidity	average acidity, balanced	average to high	average	average	average
Sweetness	slightly sweet	absent	slightly sweet	weak, slightly sweet	weak, slightly sweet
Astringence	structured, slightly bitter	velvety soft, slightly bitter, less astringent than SBControl	structured to tannic	structured to tannic	structured to tannic with bitter notes that bother and more astringent than the other samples
Extract	structured, balanced, long	more balanced compared with SBControl, but less long	structured, balanced, long, intense	structured, balanced	too extractive and thick
Colour intensity	average to high	average to high	average to high, more intensely colored than SBGSH20AA00	average to high	high to very high
Colour nuance	oxidized, spotted to light pink	greenish yellow with golden shade	greenish yellow, more oxidised than SBGSH20AA00	greenish with yellowish shade	oxidized, spotted to light pink
Total aroma intensity	low to average	average	average	average to high	low to average
Flavor description	neutral, weak floral and fruity nuance , intense shades of oxidized apple and quince	weak shades of elderflower, mint and citrus flowers, lime flowers; intense fruity shades of citrus (lemon, grapefruit, orange peel), relatively thiolic	intense shades of citronella, light shades of green cucumber and yogurt, low shades fruity melon and intense citrus nuances (lime and lemon, and then grapefruit). AA intensifies citrus note	moderate nuances of spring flowers, floral nuances and average fruity citrus nuances; more terpenic, less thiolic	weak floral nuances; weak fruity nuances (melon and citrus); average vegetal notes, oxidized, phenolic, quinonic

The same improvement of the sensory profile determined by the treatment with GSH resulted also for the Muscat Ottonel variety, but MOGSH40AA00 sample has been particularly distinguished.

In the Table 10 the characteristics' evaluation on the second tasting form for Muscat Ottonel wine samples are presented.

We can notice that the Muscat Ottonel wines had the same behavior like the Sauvignon Blanc wines. The Muscat Ottonel samples produced with GSH and AA have been evaluated also with a higher level of astringency and a bittersweet note more intense than that perceived by tasters in the samples produced only with GSH. The sample wines MOGSH20AA00 and MOGSH40AA00 have been characterized as structured, elegant, soft and velvety. Also in this case the wine sample MOGSH40AA00 was declared the best by wine tasters.

Table 10. Wine characteristics evaluation on the second tasting form for Muscat Ottonel wines

Wine characteristics	MOControl	MOGSH20AA00	MOGSH20AA50	MOGSH40AA00	MOGSH40AA50
Acidity	average to high	average to high	average to high	average to high	average to high
Sweetness	slightly sweet	slightly sweet	slightly sweet	slightly sweet	slightly sweet
Astringence	structured, slightly bitter	velvety soft	structured, AA intensifies bitter notes	structured, but velvety soft , the less bitter and astringent	structured to tannic with more pronounced bitter notes that MOGSH40AA00, but more long; AA gives corpolence to wine.
Extract	balanced	balanced	balanced	balanced, the most vinous	balanced
Colour intensity	average to high	average to high	average to high, more intensely colored than SBGSH20AA00	average to high	high to very high
Colour nuance	greenish yellow with golden shade	greenish yellow with golden shade	greenish yellow with golden shade	greenish yellow with golden shade	greenish yellow with golden shade
Total aroma intensity	average to high	average	average	average to high	average
Flavor description	weak flower shades of elderflower, linden and roses, honey; terpenic, unoxidized	weak flower shades of linden and acacia, more pronounced flower scents comparing with MOControl, smoother smell, quince jam shades	weak flower shades, vegetal notes, poorer as aroma comparing with MOGSH20AA00, but rounder taste; light aroma of green apple, but unoxidized	weak nuances of spring flowers, floral nuances and more intense apple fruity nuances, low shades toasted walnuts; terpenic, but more elegant comparing with MOGSH20AA00, beautiful citrus notes	weak floral nuances; moderate fruity nuances (citrus); low roasted nuances, more neutral than MOGSH40AA00, mineral with a certain hardness

CONCLUSIONS

All wine samples either originating from Sauvignon Blanc or Muscat Ottonel musts treated with GSH, with or without AA, led to a persistence of flavour and colour intensity higher than those reported by wine tasters for the control samples. The wines were characterized as balanced, structured and with a complex sensory profile. AA presence caused the intensification of bitter notes, both in comparison with the control samples and samples produced only with GSH. This wine damage by AA was more evident as the dose of GSH increased. Samples of wines produced only with GSH have been described as soft and velvety and received higher scores from the tasters, both compared with the controls and the wines samples produced with GSH and AA. The samples produced with a dose of 40 mg/L GSH and without AA were considered the best by tasters for both wines varieties and have received the highest scores.

In conclusion, the AA administration in grape musts seems to have less beneficial influence on the sensory profile of obtained wines from both varieties. Treatment with GSH especially the one without AA led to the achievement of better wines with more complex sensory profiles. The study clearly demonstrates that the type and the doses of antioxidants used have an important influence in terms of protecting the varietal aromas of wines. The dose of 40 mg/L GSH, higher than the approved dose by OIV of 20 mg/L for addition in musts, could be more suitable for use in the primary winemaking. This direction must be confirmed by further research that could demonstrate the benefits of treatment with GSH and may lead to the optimal dose establishment for administration in grape musts prior the inoculation with selected yeasts.

REFERENCES

Antoce O.A., 2007. Oenology. Chemistry and sensory analysis. Universitaria Publishing House, Craiova, 489-635, 667-779.

Antoce, O.A., Namolosanu, I., 2007. Methodological particularities on the application of sensory analysis to the characterisation of varietal wines. Lucrari stiintifice" Vol. 15(2), International Symposium "Accomplishments and perspectives in horticulture, viticulture, winemaking and forestry", Feb. 28 –March 3, 2007, State Agrarian University, Kyshinev, Moldova Republic, 219-223.

Badea G.A., Antoce O.A., 2015. Glutathione as a possible replacement of sulfur dioxide in winemaking technologies: a review.Scientific Papers. Series B, Horticulture, Volume LIX, ISSN 2285-5653, 123-140.

Brajkovich, M., Tibbits, N., Peron, G., Lund, C. M., Dykes, S. I., Kilmartin, P. A., et al., 2005. Effect of screwcap and cork closures on SO2 levels and aromas in a Sauvignon Blanc wine. J. Agric. Food Chem. 53(26), 10006–10011.

Cheynier, V. F., Trousdale, E. K., Singleton, V. L., Salgues, M. J., Wylde, R., 1986. Characterization of 2-S-glutathioylcaftaric acid and its hydrolysis in relation to grape wines. J. Agric. Food Chem. 34, 217–221.

Cheynier, V., Souquet, J. M., Moutounet, M., 1989. Glutathione content and glutathione to hydroxycinnamic acid ratio in Vitis vinifera grapes and musts. Am. J. Enol. Vitic. 40, 320–324.

Du Toit, W. J., Marais J., Pretorius, I. S., du Toit, M., 2006. Oxygen in must and wine: A review. S. Afr. J. Enol. Vitic. 27, 76–94.

Du Toit, W. J., Lisjak, K., Stander, M., Prevoo, D., 2007. Using LC–MSMS to assess glutathione levels in South African white grape juices and wine made with different levels of oxygen. J. Agric. Food Chem. 55, 2765–2769

Du Toit W., Obersholster A., 2014. Antioxidants in wine during fermentation. Processing and impact on antioxidants in beverages. Chapter 7, 59-67, https://www.elsevier.com/books/processing-and-impact-on-antioxidants-in-beverages/preedy/978-0-12-404738-9

Garde-Cerdán, T., Ancín-Azpilicueta, C., 2007. Effect of SO2 on the formation and evolution of volatile compounds in wines, Food Control 18, 1501–1506.

Kilmartin, P. A., 2010. Understanding and controlling nonenzymatic wine oxidation, Woodhead Publishing Series in Food Science, Technology and Nutrition, 432–458

Lavigne-Cruège, V., Pons, A., Chone, X., Dubourdieu, D., 2003. Rôle du glutathione sur l'évolution aromatique des vins blancs secs. In: 7e Symposium international d'Oenologie, Bordeaux.

Maggu, M., Winz, R., Kilmartin, P. A., Trought, M. C. T., Nicolau, L., 2007. Effect of skin contact and pressure on the composition of Sauvignon Blanc must. J. Agric. Food Chem. 55, 10281–10288.

Oliveira, C. M., Silva Ferreira, A. C., Guedes de Pinho, P., Hogg, T. A., 2002. Development of a potentiometric method to measure the resistance to oxidation of white wines and the antioxidant power of their constituents. J. Agric. Food Chem. 50(7), 2121–2124.

Park, S. K., Boulton, R. B., Noble, A. C., 2000. Formation of hydrogen sulfide and glutathione during fermentation of white grape musts. Amer. J. Enol. Vitic. 51, 91–97.

Patel, P., Herbst-Johnstone, M., Lee, S. A., Gardner, R. C., Weaver, R., Nicolau, L.,Kilmartin, P. A., 2010. Influence of juice pressing conditions on polyphenols, antioxidants and varietal aroma of Sauvignon blanc microferments. J. Agric. Food Chem. 58, 7280– 7288.

Pons, A., Lavigne, V. R., Landais, Y., Darriet, P., Dubourdieu, D., 2010. Identification of a sotolon pathway in dry white wines. J. Agric. Food Chem. 58(12), 7273–7279.

Singleton, V.L., Salgues, J., Zaya, J., Trousdale, E., 1985. Caftaric acid disappearance and conversion to products of enzymatic oxidation in grape must and wine. Amer. J. Enol. Vitic. 36, 50–56.

Singleton, V. L., 1987. Oxygen with phenols and related reactions in musts, wines, and model systems: observations and practical implications. Amer. J. Enol. Vitic. 38, 69–77.

Sonni, F., Clark, A. C., Prenzler, P. D., Riponi, C., Scollary, G. R., 2011a. Antioxidant action of glutathione and the ascorbic acid/glutathione pair in a model white wine. J. Agric. Food Chem. 59, 3940–3949.

Sonni, F., Moore, E. G., Clark, A. C., Chinnici, F., Riponi, C., Scollary, G. R., 2011b. Impact of glutathione on the formation of methylmethine- and carboxymethine-bridged (+)-catechin dimers in a model wine system. J. Agric. Food Chem. 59, 7410–7418.

Stoian V., 2011. Great book of tasting wines. Wine tasting explained for everybody, third Edition. Agricola Publishing House, Bucharest, 192-206, 244-246.

Ugliano, M., Kwiatkowski, M. J., Vidal, S., Capone, D., Siebert, T., Dieval, B., Aagaard, O., Waters, E. J., 2011. Evolution of 3-mercatohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen. J. Agric. Food Chem. 59, 2564–2572.

Vaimakis, V., Roussis, I. G., 1996. Must oxygenation together with glutathione addition in the oxidation of white wine. Food Chemistry 57, 419–422.

Walker, R., 1985. Sulphiting agents in foods: Some risk/benefit considerations. Food Additives and Contaminants 2(1), 5–24.

Webber V, Valduga Dutra S, Rodrigues Spinelli F, Rossi Marcon A,João Carnieli G, Vanderlinde R, 2014. Effect of glutathione addition in sparkling wine. Food Chemistry 159, 391–398.

***International Wine Contest Bucharest 2017, Organisation regulations, Awards, www.iwcb.ro/regulation/ accesed in January 2017

***Resolution OIV-OENO 445-2015, Treatment of wine with glutathione, approved in OIV General Assembly, in Mainz, Germany, 2015, www.oiv.int/oiv/info/enresolution?lang=en accessed in January 2016.

***Resolution OIV-OENO 446-2015, Treatment of must with glutathione, approved in OIV General Assembly, in Mainz, Germany, 2015, www.oiv.int/oiv/info/enresolution?lang=en accessed in January 2016

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ENGINEERING PROPERTIES OF THE ŞIRE GRAPE (*VITIS VINIFERA* L. CV.)

Reşat ESGİCİ[1], Gültekin ÖZDEMİR[2], Göksel PEKİTKAN[3], Konuralp ELİÇİN[3], Ferhat ÖZTÜRK[4], Abdullah SESSİZ[3]

[1]Dicle University, Bismil Vacational High School, Diyarbakır, Turkey
[2]Dicle University, Faculty of Agriculture, Department of Horticulture, Diyarbakır, Turkey
[3]Dicle University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering, Diyarbakır, Turkey
[4]Dicle University, Faculty of Agriculture, Department of Field Crops, Diyarbakır, Turkey

Corresponding author: asessiz@dicle.edu.tr

Abstract

Turkey will continue to acting an important role in grape production and raisin exportation in the world because of its large number of grape varieties, favorable ecological conditions and large amount of production areas. Turkey is the one of the gene center of grapevines, for this reason it possesses over 1600 grape varieties. Grapevine varieties are generally harvested by hand; however, the feasibility of using a mechanical harvester is some engineering properties such as physical and mechanical properties must be consideration. In this study, some physical and mechanical properties of grape berries and canes of local variety Şire (Vitis vinifera L. cv.) were determined depend on phenological stages. This research was performed at commercial vineyard in Dicle, the town of Diyarbakır, which is located in the southeastern part of Turkey. Cutting properties were measured by The Lloyd LRX plus materials testing machine. Grape berries length, width, thickness, arithmetic and geometric mean diameter, sphericity, roundness, detachment force (FDF), weight (W), the ratio of FDF/W, skin firmness, total soluble solids content, pH, total acidity and cane of grapevine shearing force, shearing strength, upper yield, shearing energy were determined. The test results indicated that very significant correlations were found between axial dimensions of grape berries, and physical dimensions, mechanical and pomological properties. The ratio of FDF/W decreased depending on phenological stages. Berry weight was lowest at the Veraison (1.60 g). The grape berry skin firmness decreased from 1.174 N to 0.766 N with phenological stages. TSSC values varied from 20.40 to 16.20 %, pH of grape (3.39-3.65) values increased with phenological stages, whereas the total acids were slight changed and reduced from 0.876 to 0.669 %. Cutting properties of Şire grapevine cane has been changed with phenological stages. Shearing force and energy requirement increased with increase internode diameter of canes. Shearing force values changed between 472.38 N and 119.57 N.

Key words: *Şire, grape berry, grape cane, physical properties, mechanical properties, engineering properties*

INTRODUCTION

Grape is an important product for the economy of Turkey. Turkey is sixth largest producer of worldwide with an estimated production of 4 million tons in 550,000 ha production area in 2016. It is the biggest exporter of raisin grapes. Each year over 200,000 tons golden coloured raisins is exported all over the world. The grape export is 170,000 tons valued at 133 million $ (Anonymous, 2016). To maintain this values and to be leading of the world's, production costs must be reduced, especially pruning, harvesting and transporting. One way of reducing production cost is use of mechanical harvesting. Mechanical harvesting of viticulture for juice is used many developed countries such as USA, France and Italia and such as country, there is valuable effort for developed and improved mechanical practices. But mechanical harvesting is not common in Turkey because grape juice sector not has improved and grape price is low, vineyards are not suitable for mechanization applications, especially in southeastern part of Turkey. Grape harvesting is made by hand. Hand harvesting is labor intensive. In fact, mechanical harvesting has not been improved and damaged product is very high. So, use of mechanization application should be increased.

Percentage presence of undamaged berries and axial dimensions are an important quality criteria both table grape and juice industry. Therefore, the economic value of grape mostly

depends on the presence of undamaged grape berries.
Mechanization of agriculture particularly harvest and after harvest has been produced big demand on the knowledge of physical and mechanical properties of products. Mechanical and physical properties of plants are important criteria in the design of machines.
The importance of understanding the physical properties of fruits is to design of machines and processes for harvesting, handling and storage of agricultural materials and for converting these materials into foods. Some of these properties include the dimensional size, shape, sphericity, bulk density, true density, porosity, geometric mean diameter, projected area, surface area, mass, volume, etc.
The knowledge related to shape and physical dimensions, is useful in sorting and sizing of fruits and determining how many fruits can place in shipping containers. These properties depend on the species, variety, diameter, maturity, moisture content and cellular structure (Mohsenin, 1986; Persson, 1987; Altuntaş and Yıldız, 2007; Nazari Galedar et al., 2008; Skubisz, 2001).
Also, the variation in the physical properties of plant branches and the resistance of cutting equipment have to be known in order to understand the behavior of material with respect to different operation of conditions.
Knowing those properties will be useful industry, academia, research Institutes, consumers, manufacturer of machines and producers of food processing equipment.
Especially, information on plant properties and the power or energy requirement of equipment has been very valuable for selecting design and operational parameters (Persson, 1987; Georget et al. 2001; Emadi et al., 2004; Voicu et al., 2011; Ghahraei et al., 2011; Hoseinzadeh and Shirneshan, 2012). Perhaps, the stem of plants cutting energy is one of the main parameters for optimizing design of cutting elements in harvesting and pruning machines (Alizadeh et al., 2011).
Therefore, comparative performance of cutting elements applied in harvester and pruning machine design can be judge by their cutting energy requirements, cutting force and stress applied (Chakraverty et al., 2003; Alizadeh et al., 2011; Sessiz et al., 2013).
Cutting strength and cutting energy are related to the stem mechanical and physical properties. Therefore, such information is very important for the suitable design of grape pruning knife and pruning machine and harvesters for efficient use of energy (Sessiz et al., 2015). With the increasing scarcity of manual labor for vineyard pruning and harvesting operations, mechanized vine pruning and harvesting has received much attention.
Mechanically harvested grapes could have as good as and sometimes better quality than hand-harvested grapes when the grapes are harvested cool and delivered promptly to the processing unit (Morris, 2000). A review of the literature revealed little information on direct cutting properties of cutting grape canes. Romano et al. (2010) determined cutting force for certain vine branches such as Cabernet, Sauvignon and Chardonnay in different regions in Italy. The tests were conducted in the laboratory and the results were processed to show if the manual forces dispensed during cutting were a function of diameters and cultivated varieties. Sessiz et al. (2015) determined cutting properties of some grape varieties in Turkey.
Studies showed that the cutting properties are valuable information for suitable design of grape pruning knifes, pruning machines and harvesters for efficient energy use. Data on physical properties of agro-food materials are valuable because they are needed as input to models predicting the quality and behavior of produce in pre-harvest, harvest situations and they aid the understanding food processing (Nesvadba et al., 2004). Therefore, to successful mechanization of grapes, we must know exactly physical and mechanical properties of grapes.
The specific objectives of this study were to: (1) determine the relationship between the basic berry and cane physical and mechanical properties of Şire grape variety in different phenological stages, (2) determine the relationship between grapevine internodes of cane's cutting properties and berry detachment force (FDF) and berry shell rupture force, (3) development of empirical model between berry axial dimensions and arithmetic, geometric mean diameter, sphericity, roundness properties, (4) to determine relationship

between phenological stages and pomological properties of grape, (5) determine the relationship between berry detachment force from cluster (FDF) and berry shell rupture force under compressive load and pomological properties of grape.

MATERIALS AND METHODS

Sample preparation and measuring

This study was performed with Şire (*Vitis vinifera* L.) local grape variety (Figure 1).
The samples were obtained from a commercial vineyard (Figure 1.) in Diyarbakır province, which is located in the southeastern part of Turkey.

Figure 1. Research vineyard area and Şire grape variety.

The grape berry and cane cutting tests were carried out during the different phenological stages of the veraison (30 August), 15 days after veraison (15 September) and harvesting time (30 Semptember) in 2016.
Grape canes which have five internode and different diameter were randomly harvested by hand from vineyard.
Harvested and collected canes which have different internode and clusters were transported to laboratory of Department of Agricultural Machinery and Technologies Engineering, University of Dicle and preservation in a refrigerator at 5 ^{0}C until the time of the cutting tests.
This study was conducted in two phases.
The first phase consist of the determination of ripening grape berries length, width, thickness, arithmetic and geometric mean diameter, sphericity, roundness, force detachment (FDF), weight (W), FDF/W, skin firmness, total soluble solids content, pH, total acidity were measured.
In the second phase, grapevine cane cutting shearing force, shearing strength, upper yield, shearing energy, specific shearing energy were determined.

Measurement of Grape Berry Axial Dimensions and Other Physical Properties

The physical properties were measured at three different phenological stages during the harvest season.
In all experiment, in order to determine the initial moisture content of grape canes, three samples of 30 g were weighed and dried in an oven of 105 ^{0}C for 24 hours (ASABE, 2006; Sessiz et al., 2007), after oven drying, samples were removed from oven and kept for 15 minutes in a desiccator for moisture equilibrium. Then samples reweighed to obtain the final moisture content using the gravimetrical method.
The weights were measured using electronic scales with a capacity of 1.2 kg and with a precision of 0.01 g. The moisture content levels of internode of cane were determined at 38.64 %, 48.00 % and, 51.76% w.b. The results were evaluated according to these moisture content values.
To determine the dimensional size of grape, 25 berrries randomly taken from four grape clusters at each phenological stage and the three linear dimensions namely, length, width and thickness were measured by using an electronic micrometer with a reading accuracy within 0.01 mm. These geometric dimensions were determined at the sample place in the middle of the berry.
The, geometric mean diameter, sphericity, roundness, and surface area of individual berries were calculated using the following equations (Mohsenin 1986; Deshpande et al., 1993; Baryeh, 2002; Aydin, 2002; Zare et al., 2012; Sessiz et al., 2013).

$$Da = \frac{(L + W + T)}{3}$$

$$Dg = (LWT)^{1/3}$$

$$\text{Ø} = \frac{(LWT)^{1/3}}{L} = \frac{Dg}{L}$$

$$Ro = \frac{W}{L} x100$$

$$S = \pi D_g^2$$

Where L is the length (mm), W is the width (mm), T is the thickness (mm), D_a is arithmetic mean diameter (mm), D_g is geometric mean diameter (mm), Ø is sphericity (%) , Ro is roundness (%), and S is surface area (mm^2).

Measurement of Grape Berry Mechanical and Pomological Properties

Grape berry detachment force, skin firmness, and the FDF/W ratio are important mechanical properties for fruit harvesting (Sessiz and Özcan, 2007; Putri et al., 2015), and firmenss is the resistance of the individual fruit to deformation under applied forces (Renny et al., 2015). These parameters were measured at three different phenological stages during the harvest period. Thickness, width and of berries were measured with a micrometer to within 0.01 mm. Grape berries were weighed by means of a digital balance with 0.01g. FDF and skin firmness were measured by using a pull digital force gauge (Model FG-20, Lutron Instrument) with stainless steel cone head adapter which apical angle 86^0. The maximum value recorded for each test, 25 grape berries were randomly selected from grape cluster and measured. The maximum skin firmness and FDF values were recorded by the force gauge while probe passing inside in grape fruit in Newton (N) (Jha et al., 2006). The digital force gauge is shown in Figure 2.

Figure 2. Force gauge

The grape berry phomological properties were measured at three different phenological stages during the harvest season. Total Soluble Solids Content (TSSC) (by refractometer), pH (by pH meter) and total acidity (bye Digital Burette) values were measured (Ozdemir et al., 2016).

Measurement of Grapevine Cane Cutting Properties

The mechanical properties the shearing force, shearing strength and shearing energy were determined along the canes from first internode to fifth internode in three phenlogical stages. Prior to the tests, the grapevine canes were cutted into five different groups (Figure 3). Five internodes of grape canes were named first to fifth from the top toward the bottom. Internode cutting locations were marked on randomly selected canes. Internodes (between two nodes were considered a internode) based on their internode of cane mean diameter ranging from 6.5 mm to 10.5 mm (6.5, 7.5, 8.5, 9.5, 10.5 mm). The cane cutting diameters were measured before the test using a caliper. The ranges of internode diameter of cane (mm) values were converted to cross-section area in mm^2. Testing was completed as rapidly as possible in order to reduce the effects of drying. All the cutting measurements were performed on the same day of harvesting.

Figure 3. Grapevine canes

The cutting tests were conducted by Lloyd LRX Plus Materials Testing Machine (Figure 4), which allows determination of the relationship between cutting strength and deformation. It has a single column with a crosshead travel range of 735 mm. In the compression tests, the test samples were placed on the machine loading table in its flat position. Loading was applied vertical direction. The cutting knife was steel, 50 mm width, 6 mm thickness and the blade angle of 17◦???. Cutting measurement were performed at 100 mm/min fixed loading speed for all tests.

Figure 4. The Lloyd LRX Plus Materials Testing Machine and cutting blade

The peak shearing strength, obtained from the shearing force findings, was determined by the following equation (Mohsenin, 1986; Beyhan, 1996; Amer Eissa et al., 2008; Zareiforoush et al., 2010; Tavakoli M., 2011; Sessiz et al., 2013):

$$\sigma s = \frac{F}{A}$$

Where: σs is the maximum shearing strength in (MPa), Fmax is the maximum shearing force in (N) and A is the cross-sectional area in (mm^2).

The cutting energy was calculated by measuring the surface area under the force-deformation curve (Yore et al., 2002; Chen, et al., 2004; Nazari Galedar, et al., 2008; Ekinci et al., 2010; Zareiforoush, et al., 2010; Heidar and Chegini, 2011; Sessiz et al., 2015; Nowakowski, 2016). The cutting energy and displacement was calculated by material testing machine. A computer data acquisition system recorded all the force-displacement curves during the cutting process. A typical force-deformation curve for grapevine cane under compression is shown in Figure 5.

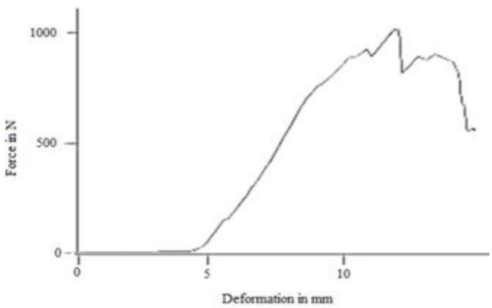


Figure 5. Typical force-deformation curve

The first peak corresponds to the yield point at which cane damage was initiated. The second peak corresponds to maximum compressive force. This bio yield point is characterized by the fact that any further compression yields no increase in applied load (Mohsenin, 1986; Lu and Siebenmorgen, 1995).

Data Analysis

The experiment was planned as a completed randomized plot design, and, data were analyzed using The General Linear Model (GLM). Mean separations were made for significant effects with LSD and the means were compared at the 1% and 5% levels of significance using the Tukey multiple range tests in JMP software, version 11.

RESULTS AND DISCUSSION

Grape Berry Properties Axial Dimensions and Physical Properties

The physical properties of grape berries at different phenological stages are presented in Table 1. The comparison of means indicated that there was a significant difference ($p<0.05$) among phenological stages values. The results indicated that the data obtained from the measured values gave the following significant correlations between axial dimensions and other physical properties such as arithmetic mean diameter, geometric mean diameter, surface area, sphericity and roundness. The axial dimensions of grape berries increased significantly ($p<0.05$) over the period of maturity time (the harvest season being usually prolonged for some 20-30 days). Dimensions of Șire grape fruits varied from 14.03 to 16.16 mm in length, 12.94 to 15.51 mm in width, and 12.78 to 15.51 mm in thickness, with average values of 15.32, 14.28, and 14.36 mm, respectively. These dimensions can be used in design of sorting and separating machine and food industry for grape. Similar results were reported by Khodaei ve Akhijahani (2012) for Rasa grape variety. Also, results show that arithmetic mean diameter, geometric mean diameter, surface area, sphericity and roundness of grape berries increases with along phenological stages ($P<0.05$).

Table 1. Axial dimensions and physical properties of Şire grape berries at different phenological stages

Phenological Stages	Dimensions (mm)			Arithmetic Mean Diameter (mm)	Geometr Mean Diameter (mm)	Area (mm^2)	Sphericity (%)	Roundness (%)
	Length (mm)	Width (mm)	Thickness (mm)					
Veraison	14.03b[1]	12.94c	12.78c	13.24c	12.22c	551.4c	0.943c	0.921b
15 days after Veraison	15.78a	14.49b	14.80b	15.09b	15.08b	716.7b	0.950b	0.930b
Harvest	16.16a	15.43a	15.51a	15.70a	15.69a	776.2a	0.971a	0.955a
Mean	15.32	14.28	14.36	14.67	14.33	681.43	0.954	0.935
LSD	0.385	0.378	0.414	0.362	0.362	33.91	0.01	0.01

[1]means followed by the same letter in each column are not significantly different by Tukey's multiple range test at the 5% level

The relationship between axial dimensions and the other physical properties can be calculated by the regression equation shown in Table 2. The high correlation was found between axial dimensions and the other physical properties values. So, the equations can be used to predict the arithmetic mean diameter, geometric mean diameter, surface area, sphericity and roundness of Şire grape variety as a function of axial dimensions and maturity time.

Table 2 . Regression equations of cutting properties as a function of three axial dimensions[1]

Parameters	Regression equation	R^2
Arithmetic mean diameter (mm)	$Y= -1.7x\ 10^{-14} +0.333L+0.333\ W+0.333\ T$	1.00
Geometric mean diameter (mm)	$Y=-0.00204 +0.3174L+0.339\ W +0.343\ T$	0.999
Surface area (mm2)	$Y= -660+29.06\ L +30.98\ W+31.43T$	0.999
Sphericity (%)	$Y= 0.9548-0.04187\ L+0.02237\ W+0.0224\ T$	0.994
Roundness (%)	$Y=0.934-0.06127\ L+0.0654\ W+0.000171T$	0.995

[1]L: Length (mm), W: Width (mm), T: Thickness

Mechanical and Pomological Properties

The values of obtained from the test results the berry detachment force from grape cluster, berry weights, FDF/W ratio and, berry shell firmness are shown in Table 3. As shown in Table 3 very high correlation was observed between the ratio of FDF/W and phenological stages. The ratio of FDF/W decreased depending on phenological stages and maturity time. Fruit weight was lowest at the beginning of the harvest (1.60 g). The fruit detachment force from the grape cluster stalk was reduced from 1.57 N to 1.27 N. This value is valuable for maturity criteria of grape fruit because FDF/W ratio is an important parameter of mechanical harvesting. Similar results were observed between skin firmness and phenological stages. The grape berry skin firmness decreased from 1.17 N to 0.766 N with harvesting period. The reason for this trend is that the water content of fruit increased with maturity. According to these results we can express that there is a high correlation between FDF/W ratio and fruit skin firmness. The maximum firmness was observed as 1.174 N when the TSSC was 16.20 at the first date of harvest (Table 3 and Table 4). Total Soluble Solids Content (16.20 - 20.40 %) and pH (3.39-3.65) values increased with harvesting date, whereas the total acidity were slight changed and reduced from 0.876 to 0.669 %. Similar results were reported by Ozdemir et al. (2016) for different wine grape variety.

Table 3. Some mechanic properties of Şire grape berries at different phenological stages

Phenological Stages	Properties			
	Detachment Force (FDF), N	Weight (W), g	(FDF/W), N/g	Skin firmness(N)
Veraison	1.57	1.60	0.98	1.174
15 days after Veraison	1.51	2.13	0.84	0.859
Harvest	1.27	2.49	0.51	0.766

Table 4. Some pomological properties of Şire grape berries at different phenological stages

	Properties			
Phenological Stages	TSSC (%)	pH	Acidity (%)	Maturit Index
Veraison	16.20	3.39	0.876	18.43
15 days after Veraison	18.23	3.50	0.775	23.52
Harvest	20.40	3.65	0.669	30.49

Grapevine Cane Cutting Properties

The test results of the cutting properties are shown in Table 5. As shown in the table, the phenological stages has significant effect on the cutting properties of grapevine canes (*P* <0.01). It can be seen from table 5 that the shearing force, shearing strength, upper yield, energy requirement and specific cutting energy has increased depending on phenological stages. There was no significant difference between the mean shearing strength for phenological stages. However, there was a significant difference between strength for internodes' diameter of cane (Table 5). It was observed that the minimum values cutting force, cutting strength, upper yield, energy requirement and specific cutting energy were obtained at a date of 30.08.2016 as 649.78 N, 13.29 MPa, 588.8, 5.02 J and 0.0944 J.mm^{-2}, while maximum values of were obtained at date of 23.09.2013 as 823.16 N, 16.11 MPa, 723.4 N, 6.25 J, and 0.1049 J mm^{-2}, respectively.

Table 5. The average cutting properties and phenological stages

Phenological Stages	Shearing force (N)	Shearing strength (Nmm^{-2})	Upper Yield (N)	Shearing energy (Joule)
Veraison	649.78^{b1}	13.29	588.8^{b}	5.02^{b}
15 days after Veraison	819.48^{a}	15.87	680.2^{ab}	6.69^{a}
Harvest	823.16^{a}	16.11	723.4^{a}	6.25^{a}
Mean	764.14	14.95	664	5.99
LSD	78.59	ns	0.098	1.13
R^2	0.941	0.575	0.886	0.872
Upper value	853.75	16.23	744	6.86
Lower value	674.53	13.67	584	5.12
Std dev	298.28	4.262	4.0	2.90
Std err mean	44.46	0.635	26.8	0.43

[1]means followed by the same letter in each column are not significantly different by Tukey's multiple range test at the 5 % level.

The results of the cutting properties depending on diameter of internodes of grapevine cane are shown in Table 6. The results shown in Table 6 indicate that the shearing force, shearing strength, upper yield, shearing energy and specific shearing energy requirement increased with increase internode diameter of canes. The significant differences were found between all of internodes of diameter at a 5 % probability level. The maximum values of shearing force shearing, upper yield and energy were obtained at 86.54 mm^2 cross-sectional area as 1.197 N, 1.060 N, and 10.16 J, respectively, while the maximum shearing strength was obtained at 70.84 mm^2 cross-section area (9.5 mm^2 diameter) as 19.675 MPa. Especially, the cross-sectional area has a significant influence on cutting properties and energy. The shearing energy values varied from 3.509 J to 10.16 J depend on diameter. The effect of stem diameter on the maximum cutting force and cutting energy is consistent with Chen et al. (2004), who reported that both the cutting energy and maximum cutting force are directly proportional to the cross-sectional area of hemp stalk. Similar results were found by Sessiz at al. (2013) for the olive sucker and Sessiz et al (2015) for grape sucker.

Table 6. The relationship between average cutting properties and cross-sectional area

Cross-sectional area (mm^2)	Shearing force (N)	Shearing strength (Nmm^{-2})	Upper Yield (N)	Shearing energy (Joule)
33.16 (6.5)[2]	472.38d[1]	14,245b	432.0 c	3,509c
44.15 (7.5)	626.45c	14,189b	540.4bc	5,004bc
56.71 (8.5)	726.89bc	12,817b	601.7b	5,092bc
70.84 (9.5)	798.38b	19,675a	686.2b	6,192b
86.54 (10.5)	1196.57a	13.82b	1,060a	10.16a
Mean	764.14	14.95	664.06	5.99
LSD	119.38	Ns	14.97	1,719

[1]means followed by the same letter in each column are not significantly different by Tukey's multiple range test at the 5 % level.

[2] diameter of internode (mm)

CONCLUSIONS

The tests results indicated that the data obtained from the measured values gave the significant correlations between axial dimensions and other physical properties such as arithmetic mean diameter, geometric mean diameter, surface area, sphericity and roundness. Dimensions of Şire grape berries varied from 14.03 to 16.16 mm in length, 12.94 to 15.51 mm in width, and 12.78 to 15.51 mm in thickness, with average values of 15.32, 14.28, and 14.36 mm, respectively. These dimensions can be used in design of sorting and separating machine and food industry for grape.

The results show that arithmetic mean diameter, geometric mean diameter, surface area, sphericity and roundness of grape fruits increases with along phenological stages ($P<0.05$). Very high correlation was observed between the ratio of FDF/W and phenological stages. The ratio of FDF/W decreased depending on phenological stages and maturity time. Fruit weight was lowest at the beginning of the harvest (1.60 g).

The grape berry skin firmness decreased from 1.17 N to 0.766 N with harvesting period. TSSC values varied from 20.40 to 16.20.

Total soluble solids content (16.20-20.40 %) and pH (3.39-3.65) values increased with harvesting date, whereas the total acids were slight changed and reduced from 0.876 to 0.669 %. Cutting properties of sire grape cane has been changed with harvesting time. Shearing force and energy requirement increased with increase internode diameter of canes. Shearing force values varied between 472.38 N and 1,196.52 N.

The maximum shearing force and energy requirement were determined the last harvesting time.

REFERENCES

Anonymous, 2016.Turkish Statistical Institute Agriculture Databases. http://www.turkstat.gov.tr/PreTabloArama.do?metod =search&araType=vt

ASABE Standards, 2006. S358.2: 1:1 Measurement – Forages. 52nd edn. American Society of Agricultural Engineers, St Joseph MI.

Altuntas E., Yildiz M., 2007. Effect of moisture content on some physical and mechanical properties of faba bean (*Vicia faba* L.) grains, J. Food Eng., vol. 78, pp.174-183, 2007.

Alizadeh M.R., F.R.Ajdadi, Dabbaghi A, 2011. Cutting energy of rice stem as influenced by internode position and dimensional characteristics of different varieties. *AJCS* 5(6):681-687, ISSN:1835-2707.

Amer Eissa A.H., Gomaa A.H,. Baiomay M.H., Ibrahim A.A, 2008. Physical and mechanical charactesristics for some agricultural residues. Journal of Agricultural Engineering, 25(1),121-146. ASAE Standards, 2006. S358.2: 1:1 Measurement –Forages. 52nd ed. American Society of Agricultural Engineers, St Joseph MI

Aydin C., 2002. Phsical properties of hazel nuts. Biosystems engineering. 82(3), 297-303

Baryeh E.A., 2002. Physical properties of millet .Journal of Food Engineering 51, 39-46.

Beyhan M.A., 1996. Determination of shear strength of hazelnut sucker. Journal of Agriculture Faculty OMU,11(3),167-181.

Chakraverty A., P.R Singh, Raghavan G.S.V., Ramaswamy H.S., 2003. Handbook of postharvest technology. 1st edn, Marcel Dekker Inc, New York.

Chen Y., Gratton J.L., Liu J. 2004. Power requirements of hemp cutting and conditioning. Biosystems Engineering, 87(4), 417–424.

Deshpande S.D, Bal S., Ojha T.P., 1993. Physical properties of soybean. Journal of Agricultural Engineering Research, 56, 89-98

Georget D.MR., Smith A.C., Waldron K.W.,2001. Effect of ripening on the mechanical properties of Portuguese and Spanish varieties of olive (*Olea europaea* L) J Sci Food Agric 81:448-454.

Ekinci K., Yilmaz D., Ertekin C., 2010. Effects of moisture content and compression positions on mechanical properties of carob pod (*Ceratonia siliqua* L.). In African Journal of Agricultural Research, vol. 5, 2010, no. 10, pp. 1015–1021.

Emadi B., Kosse V., Yarlagadda P., 2004. Relationship between mechanical properties of pumpkin and skin thickness. International Journal of Food Properties, 8(2), 277-287.

Ghahraei O., Ahmad D., Khalina A., Suryanto H.,Othman J. 2011. Cutting tests of kenaf stems. Transactions of the ASABE, 54(1), 51-56.

Heidari A., Chegini G.R.,2011. Determining the shear strength and picking force of rose flower. Agricultural Engineering. Ejpau 14(2), 13.

Hoseinzadeh B., Shirneshan A. 2012. Bending and shearing characteristics of canola stem. American-Eurasian J. Agric. & Environ.Sci., 12 (3), 275-281.

Jha S.N. , Kingsly A.R.P., Sangeeta C,. 2006. Physical and mechanical properties of mango during growth and storage for determination of maturity. Journal of Food Engineering,73–76.

Khodaei J., Akhijahani H.S.,2012. Some physical properties of Rasa grape (*Vitis vinifera* L.).World Applied Sciences Journal 18 (6): 818-825

Lu R., Siebenmorgen T. J.,1995. Correlation of head rice yield to selected physical and mechanical properties of rice kernels. Transactions of the ASAE .VOL. 38(3):889-894.

Mohsenin N.N. ,1986. Physical properties of plant and animals materials. 2nd edition. New York, NY: Gordon and Breach Science Publishers.

Morris J.R. ,2000. Past, Present, and future of vineyard mechanization. Proceeding ASEV 50 th Anniv. Ann. Mtg. Seatle, WA, Vol. 51, 155-164.

Nazari G.M., Tabatabaeefar A., Jafari A., Sharifi A., Rafiee S., 2008. Bending and shearing characteristics of alfalfa stems. Agricultural Engineering International the CIGR Ejournal. Manuscript FP 08 001. Vol. X. May.

Nesvadba N., Houska M., Wolf W., Gekas V., Jarvis D., Sadd P.A., johns A.I., 2004. Database of physical properties of agro-food materials. Journal of Food Engineering 61, 497-503.

Nowakowski T. ,2016. Empirical model of unit energy requirements for cutting giant miscanthus stalks depending on grinding process parameters. Annals of Warsaw University of Life Sciences – SGGW, Agriculture No 67 (Agricultural and Forest Engineering) 2016: 63–70.

Ozdemir G., Sogut A.B., Pirinccioglu M., Kizil G., Kizil M.,2016. Changes in the phytochemical components in wine grape varieties during the ripening period. Scientific Papers B, Horticulture Vol. LX, 85-93.

Persson S., 1987. Mechanics of cutting plant material. ASAE Publications, St Joseph, MI, USA

Putri R., Yahya E., Adam A., Aziz N.M., Samsuzana, A.A.,2015. Correlation of moisture content to selected mechanical Properties of Rice Grain samples. International Journal on Advanced Science Engineering Information Technology. Vol.5, ISSN: 2088-5334.

Romano E., Bonsignore R., Camillieri D., Caruso L., Conti A., Schillaci G.,2010. Evaluation of hand forces during manual vine branches cutting. International Conference Ragusa SHWA, September 16-18, 2010 Ragusa Ibla Campus- Italy. Work Safety and Risk Prevention in Agro-food and Forest Systems.

Renny E.P., Yahya A., Adam N.M., Aziz, Samsuzana, A.A.,2015. Correlation of moisture content to selected mechanical properties of rice grain samples. (2015). International Journal on Advanced Science Engineering Information Technology. Vol.5, ISSN: 2088-5334.

Sessiz A., Özcan M.T., 2006. Olive removal with Pneumatic branch shaker and abscission chemical .Journal of Food Engineering, Volume: 76 Issue: 2 Pages: 148-153.Elsevier, London.

Sessiz A., Esgici R., Kızıl S., 2007. Moisture-dependent physical properties of caper (*Capparis* ssp.) Fruit. Journal Of Food Engineering", 79, 1426-1431. Elsevier, London.

Sessiz A., Elicin A.K., Esgici R., Ozdemır G., Nozdrovıcký L., 2013. Cutting properties of olive sucker. Acta Technologica Agriculturae. The Scientific Journal for Agricultural Engineering, The Journal of Slovak University of Agriculture in Nitra. Vol: 16(3), 80–84.

Sessiz A., Esgici R., Özdemir G., Eliçin A.K., Pekitkan F.G., 2015. Cutting properties of different grape varieties, Agriculture & Forestry, Vol. 61. Issue 1: 211-216.

Skubisz G., 2001. Development of studies on the mechanical properties of winter rape stems. International Agrophysics, 15,197-200. Taghijarah H., Ahmadi H., Ghahderijani M.

Tavakoli M., 2011. Shearing characteristics of sugar cane (*Saccharum officinarum* L.) 157 stalks as a function of the rate of the applied force. AJCS 5(6), 630-634.

Voicu G., Moiceanu E., Sandu M., Poenaru I.C., Voicu P., 2011. Experiments regarding mechanical behaviour of energetic plant miscanthus to crushing and shear stress. *Engineering For Rural Development Jelgava*, 26.- 27.05.2011.

Yore M.W., Jenkins B.M, Summers M.D., 2002. Cutting properties of rice straw. *Paper Number: 026154.*ASAE Annual International Meeting / CIGR XVth World Congress

Zare D., Salmanizade F., Safiyari H.,2012. Some Phyical and mechanical properties of Russian olive fruit. World Academiy of Science, Engineering and Technology. International Journal and biological, Biomolecular, Agricultural, Food and Biotechnologicval Enginerring. Vol:6(9), 668-671.

Zareiforoush H., Mohtasebi S.S., Tavakoli H., Alizadeh M.R., 2010. Effect of loading rate on mechanical properties of rice (*Oryza sativa* L.) straw. Australian Journal of Crop Science, 4(3), 190–195.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PROTECTIVE EFFECT OF ÖKÜZGÖZÜ (*VITIS VINIFERA* L. CV.) SEED EXTRACT AGAINST HYDROXYL RADICAL INDUCED DNA DAMAGE

Mihdiye PİRİNÇÇİOĞLU[1], Göksel KIZIL[1], Bircan ÇEKEN TOPTANCI[1], Gültekin ÖZDEMIR[2], Murat KIZIL[1]

[1]Dicle University, Faculty of Science, Department of Chemistry, Diyarbakir, Turkey
[2] Dicle University, Faculty of Agriculture, Department of Horticulture, Diyarbakir, Turkey

Corresponding author email: gozdemir@dicle.edu.tr

Abstract

Grape is one of the most commonly consumed fruits in the world. It has various biological functions, due to its potential of rich polyphenol ingredients, most of which are contained in its seeds (70%) and skin (30%).The aim of this study was to investigate the protective role of Öküzgözü cv. grape seed extracts against hydroxyl radical that induced oxidative DNA damage. The results revealed that the presence of various concentrations of grape seed extract in the reaction mixture significantly inhibited DNA damage induced by reactive oxygen species (ROS). In conclusion, the results demonstrated that Öküzgözü cv. grape seed extracts protected DNA against hydroxyl radical that induced oxidative damage and the extract could be used as a valuable food supplement or a nutraceutical product.

***Key words**: grape, Öküzgözü, DNA damage, hydroxyl radical.*

INTRODUCTION

The opinion of cancer prevention through antioxidant intervention is due to the fact that fruits, vegetables and plants contain antioxidants and are associated with low cancer rates in those who consume them (Collins, 2005).
The protective effect of plants against DNA damage and protein oxidation can be demonstrated *in vitro*. A growing number of natural components of food, particularly fruits and vegetables are regarded as possible antioxidants with a role in protecting the cell against free radicals damage and chemicals, which can generate the oxidative forms (Collins et al., 2001). Several substances in plants express cytotoxic and genotoxic activities and shown correlation with the incidence of tumours (Srividya et al., 2013). Therefore, understanding of the health benefits and/or potential toxicity of these plants is important.
Grape is one of the most widely grown fruit crops throughout the world. Grape is the world's largest fruit crop, with more than 61 million metric tons, cultivated mainly as *Vitis vinifera* for wine production (Arvanitoyannis, 2006). *Vitis vinifera* (common grape vine) is a species of *Vitis*, native to the Mediterranean region, central Europe, and southwestern Asia, from Morocco and Portugal north to southern Germany and east to northern Iran (Gazioğlu Sensoy, 2012).
Turkey, today, is the fifth largest producer of grapes and is becoming one of the most important wine producers in the world. Öküzgözü is a Turkish grape variety and Turkish wine is produced from this grape. Öküzgözü is a rounded, dark red grape and is the largest among the grape varieties grown in Turkey.
Previous reports show that the leaves, fruits and juice of *V. vinifera* L. have a hepatoprotective effect on acetaminophen induced hepatic DNA damage, apoptosis and necrotic cell death (Pirinççioğlu et al., 2012).
The antioxidant activity of grape seed extract which were prepared using various solvents, such as acetone, ethyl acetate, methanol and mixtures of different solvents, such as ethyl acetate (EtOAc) and water were evaluated by using a β-carotene-linoleate model system and linoleic acid peroxidation method. At 100 ppm concentration, various extracts showed 65–90% antioxidant activity (Jayaprakasha et al., 2001).

In another study anti-hyperglycaemic and antioxidant effect of grape seed extract was investigated in normal and streptozotocin-induced diabetic Wistar rats.

The results showed that oral administration of grape seed extract (100 mg/kg/day) reduced the levels of lipid peroxides and carbonylated proteins and improved the antioxidant activity in plasma and hepatic tissue in rats treated with grape seed natural extract as compared with the diabetic control rats.

These results suggested that the grape seed extract enhanced the antioxidant defence against reactive oxygen species produced under hyperglycaemic conditions, hence protecting the liver cells (Chis et al., 2009).

The main objective of this study was to investigate the protective role of Öküzgözü cv. grape seed extracts against hydroxyl radical that induced oxidative DNA damage.

MATERIALS AND METHODS

Plant Material

Fresh Öküzgözü cv. grape samples were harvested at optimum maturity from Elazığ (Sün Village).

After removing the seeds by squeezing the berry gently by hand, grapes (2 kg) were washed and the seeds were removed from the pulp by squeezing the fruit gently (Figure 1).

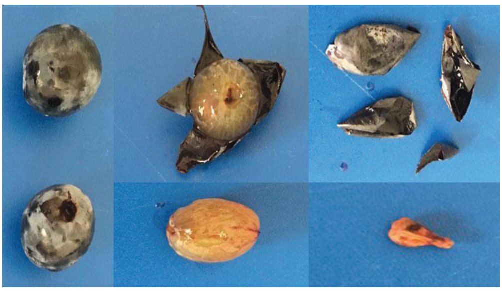


Figure 1. Öküzgözü cv. grape berry

Seeds were dried at room temperature and powdered in a blender. Powdered seeds were extracted with petroleum ether (250 mL) at 60 ^{o}C for 6h and ethyl acetate:methanol:water (150:75:25; 250 mL) for 8 h in a Soxhlet extractor, respectively. Finaly, ethyl acetate extract was filtered under pressure, then the filtrate was frozen and lyophilised in a lyophiliser (Martin Christ, 0.21 mm Hg, 80 °C) for 24 h. The lyophilised powder of seeds (4.5 g) was stored at -20°C until use (Baydar et al., 2007).

DNA cleavage protective effect of Öküzgözü cv. grape seed extract

DNA damage protective effect of Öküzgözü cv. grape seed extractagainst hydroxyl radical that induced DNA damage was investigated on pBluescript M13 (+) plasmid DNA. Plasmid DNA was isolated by Qiagene plasmid miniprep kit (Kızıl et al., 2003). Plasmid DNA was oxidized with H_2O_2 + UV treatment in presence of seed extract and checked on 1% agarose after modification (Attaguile et al., 2000). In brief, the experiments were performed in a volume of 10 µL in a microcentrifuge tube containing 200 ng of plasmid DNA in phospate buffer (7.14 mmol phospate and 14.29 mmol NaCl), pH 7.4. H_2O_2 was added at a final concentration of 2.5 mmol/L with and without 1 µL of (100, 200, 300, 400 µg/mL) seed extract. The reactions were initiated by UV irradiation and continued for 5 min on the surface of a UV transilluminator (8000 µW cm^{-1}) at 300 nm at room temperature. After irradiation, the reaction mixture (10 µL) with gel loading dye was placed on 1% agarose gel for electrophoresis. Electrophoresis was performed at 40 V for 3 h in the presence of ethidium bromide (10 mg/mL). Untreated pBluescript M13+ plasmid DNA was used as a control in each run of gel electrophoresis along with partial treatment, i.e., only UV treatment and only H_2O_2. Percent inhibition of the DNA strand scission was calculated as follows.

$$\text{Inhibition (\%)} = I-[(S_{m+a}-S_c)/(S_m-S_c)]$$

where S_{m+a},is the percentage remaining supercoiled after treatment with mix plus agent, S_c, is the percentage remaining supercoiled in control untreated plasmid and S_m, is the percentage remaining supercoiled with mix without agent (Fukuhara et al., 1998).

Densitometric analysis of treated and control pBluescript M13+ plasmid DNA

Gel was scanned on Gel documentation system (Gel-Doc-XR, BioRad, Hercules, CA, USA).

Bands on the gels were quantified discovery series Quantity One programme (version 4.5.2, BioRad Co.).

RESULTS AND DISCUSSIONS

When DNA was exposed to H_2O_2 and irradiated with UV light, H_2O_2 will generated to hydroxyl radicals, then the supercoiled form of DNA would cleave. Figure 2 shows the quantified band intensity for the sc-DNA (form I), oc-DNA (form II) and l-DNA (form III) and the electrophoretic pattern of DNA after UV-photolysis of H_2O_2 (2.5 mM) in the absence and presence of the seed extract of *V. vinifera* L. (100, 200, 300, 400, 500 µg/mL). DNA derived from pBluescript M13+ DNA plasmid showed two bands on agarose gel electrophoresis (lane 1), the faster moving band corresponded to the native form of supercoiled circular DNA (scDNA) and the slower moving band was the open circular form (ocDNA). The UV irradiation of DNA in the presence of H_2O_2 (lane 2) resulted in the cleavage of scDNA to linear form (linDNA), indicating that $^{\bullet}$OH radical generated from UV photolysis of H_2O_2 produced DNA strand scission. The addition of extract (lanes 6-10) to the reaction mixture suppressed the formation of linDNA and induced a partial recovery of scDNA. In fact, the intensity of scDNA bands scanned from the agarose gel electrophoretic patterns was 45.69, 51.07, 61.77 and 68.07 % for plasmid DNA treated with H_2O_2 in the presence of 100, 200, 300, 400, and 500 µg/mL extract, respectively, as compared with the untreated plasmid DNA. The inhibition activities of *V. vinifera* L. seed extract on DNA damage were found to be between 18.10-62.69% at the concentrations rate of 100-500 µg/mL.

Thus, the identification of natural products able to provide protection against UV radiation-induced inflammatory responses and the generation of oxidative stress may have important human health implications. In fact, seed extract suppressed the formation of linDNA, generated by exposure of plasmid DNA to OH radical generated by H_2O_2 UV-photolysis, and induced a partial recovery of scDNA. DNA damage protecting activity of *V. vinifera* L. seed extract is corresponding to its antioxidant potential.

ROS-induced DNA damage can be described both chemically and structurally and shows a characteristic pattern of modification. It is well known that in various cancer tissues free radical-mediated DNA damage was found (Valko et al., 2001). The majority of these changes can be reproduced by ROS experimentally including the following: modification of all bases, production of base-free sites, deletions, frameshifts, strand breaks, DNA-protein cross-links, and chromosomal rearrangement. An important reaction involved in DNA damage involves generation of hyrodxyl radical, e.g., through Fenton chemistry (Brezova et al., 2003). Hydroxyl radical is known to react with all components of the DNA molecule: the purine and primidine bases as well as the deoxyribose backbone (Valko et al., 2004).

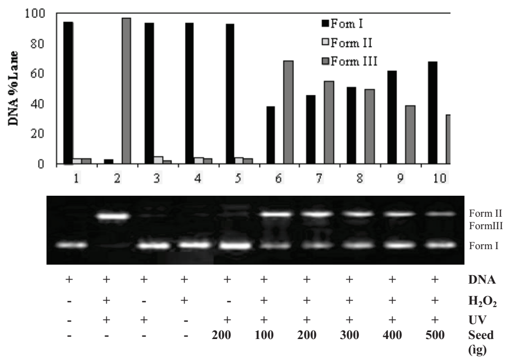


Figure 2.The quantified band intensity for the sc-DNA (form I), oc-DNA (form II) and l-DNA (form III) with Quantity One 4.5.2. version software and Electrophoretic pattern of pBluescript M13+ DNA oxidized with H_2O_2+UV treatment in presence of seed extract

Reaction vials contained 200 ng of supercoiled DNA (31.53 nM) in distilled water, pH 7). Electrophoresis was performed using 1% agarose at 40 V for 3 h in the presence of ethidium bromide (10 mg/mL).

Electrophoresis running buffer: TAE (40 mM Tris acetate, 1 mM EDTA, pH 8.2). Lane 1, control DNA; Lane 2, DNA + H2O2 (2.5 mM)+UV; Lane 3, DNA + UV; Lane 4, DNA + H2O2 (2.5 mM); Lane 5, DNA + Vv (200 µg/mL); Lane 6, DNA + Vv (100 µg/mL) + H2O2 (2.5 mM) + UV; Lane 7, DNA + Vv (200 µg/mL) +H2O2(2.5 mM) + UV; Lane 8, DNA + Vv (300 µg/mL) + H2O2 (2.5 mM)+UV; Lane 9, DNA + Vv (400 µg/mL) +

H_2O_2 (2.5 mM) + UV; Lane 10, DNA + Vv (500 µg/mL) + H_2O_2 (2.5 mM) + UV. Reactions were all performed at room temperature in phospahate buffer containing 100 mM sodium chloride.

CONCLUSIONS

The present study suggests that, grape seed extract is capable of suppressing DNA cleavage *in vitro*. Therefore, may be beneficial in the prevention of reactive oxygen species (ROS) related diseases, such as cardiovascular, inflammatory and cancer.
In conclusion, we still do not know how fruits, vegetables and plants protect against cancer, but it seems increasingly unlikely that it is simply because they contain high concentrations of antioxidants.

REFERENCES

Arvanitoyannis I.S., Ladas D., Mavromatis A. 2006. Wine waste treatment methodology. International Journal of Food Science & Technology, 41:10, 1117-1151.

Attaguile G., Russo A., Campisi F., Savoca F., Acquaviva R., Ragusa N., Vanella A., 2000. Antioxidant activity and protective effect on DNA cleavage of extracts from *Cistus incanus* L. and *Cistus monspeliensis* L. Cell Biology and Toxicology, 16: 83-90.

Brezova V., Valko M., Breza M., Morris H., Telser J., Dvoranova D., Kaiserova K., Varecka L., Mazur M., Leibfritz D., 2003, Role of radicals and singlet oxygen in photoactivated DNA cleavage by the anticancer drug camptothecin: An electron paramagnetic resonance study. The Journal of Physical Chemistry B, 107: 2415-2425.

Baydar N.G., Özkan G., Yaşar S., 2007. Evaluation of the antiradical and antioxidant potential of grape extracts. Food Control, 18:1131-1136.

Chis I.C., Ungureanu M.I., Marton A., Simedrea R., Muresan A., Postescu I.D., Decea N., 2009. Antioxidant effects of a grape seed extract in a rat model of diabetes mellitus.Diabetes and Vascular Disease Research, 6(3):200-204.

Collins B.H., Horská A., Hotten P.M., Riddoch C., Collins A.R., 2001. Kiwifruit protects against oxidative DNA damage in human cells and in vitro.Nutrition and Cancer, 39: 148–153.

Collins A.R., 2005. Antioxidant intervention as a route to cancer prevention. European Journal of Cancer Nutrition and Cancer, 41(13):1923-1930.

Fukuhara K., Miyata N., 1998. Resveratrol as a new type of DNA-cleaving agent. Bioorganic Medicinal Chemical Letters, 8: 3187-3192.

Gazioğlu Sensoy R.İ., 2012. Determination of Phenolic Substances and Antioxidant Activities in Some Grape Cultivars by HPLC. The Journal of Animal & Plant Sciences, 22(2): 2012, Page: 448-451.

Jayaprakasha G.K., Singh R.P., Sakariah K.K., 2001. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chemistry, 73: 3, 285-290.

Kızıl M., Yilmaz E.I., Pirinccioglu N., Aytekin Ç., 2003. DNA cleavage activity of diazonium salts: Chemical nucleases. Turkish Journal of Chemistry, 27:539-544.

Montvale N.J.,2002. PDR for Herbal Medicines, second ed. Med Econ, 362-363.

Pirinççioğlu M., Kızıl G., Kızıl M., Özdemir G., Kanay Z., Ketani M.A. 2012. Protective effect of Öküzgözü (*Vitis vinifera* L. cv.) grape juice against carbon tetrachloride induced oxidative stress in rats. Food Function, 3(6): 668-673.

Srividya A.R., Dhanbal S.P., Sathish Kumar M.N., Vishnuvarthan V.J., 2013. Genoprotective activity of hydro alcoholic extract of Curcuma aromatica and Curcuma zedoaria rhizomes and their comparison with Curcumin. The Journal of Ethnobiology and Traditional Medicine, 306-321.

Valko M., Mario I., Mazur M., Rhodes C.J., Telser J., 2004. Role of oxygen radicals in DNA damage and cancer incidence. Molecular and Cellular Biochemistry, 266: 37-56.

Valko M., Morris H., Mazur M., Rapta P., Bilton R.F.2001. Oxygen free radical generating mechanisms in the colon: Do the semiquinones of vitamin K play a role in the aetiology of colon cancer? Biochimica et Biophysica Acta, 1527:161-166.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRECISION VITICULTURE TOOLS TO PRODUCTION OF HIGH QUALITY GRAPES

Gultekin OZDEMIR[1], Abdullah SESSIZ[2], Fatih Goksel PEKITKAN[2]

[1]Dicle University, Faculty of Agriculture, Department of Horticulture, Diyarbakir, Turkey
[2]Dicle University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering, Diyarbakir, Turkey

Corresponding author email: gozdemir@dicle.edu.tr

Abstract

Grapes are the most widely grown commercial fruit crop in the world, and also one of the most popular fruit crops for horticultural production. Grape growers constantly search the ways in order to maximize their profits all over the world. It becomes to be important to use new information technologies to increase to overall returns. Precision Viticulture (PV) refers to the application of new and emerging information technologies to the production of grapes to improve the efficacy of production, maximize the quality of production, minimize the environmental footprint of production and minimize the risk associated with production for the grower and processer. Precision viticulture depends on new and emerging technologies such as global positioning systems (GPS), meteorological and other environmental sensors, satellite and airborne remote sensing, and geographic information systems (GIS) to assess and respond to variability. It can be possible that take under control such as soil fertility, fertilizer application norm, disease, water, weed, harvesting, and environmental management by precision viticulture systems in vineyard. So, to reduce inputs such as fertilizer, water, pesticides and to increase yield and quality of grape berries, we must to increase precision technologies in our vineyards. In this review, Precision Viticulture tools will be demonstrated to producing of high quality grapes. Finally, this study will also help grape growers and government agencies that provide new information and technologies such as Remote Sensing to growers in order to detect some factors affecting to maximize grape production.

Key words: *Geographical Information Systems, Grape, Precision Viticulture, Remote Sensing, Vineyard Management.*

INTRODUCTION

Precision viticulture is precision farming applied to optimize vineyard performance, maximizing grape yield and quality while minimizing environmental impacts and risk (Proffitt et al., 2006; Urretavizcaya et al., 2017). This is accomplished by measuring local variation in factors that influence grape yield and quality (soil, topography, microclimate, vine health, etc.) and applying appropriate viticulture management practices (trellis design, pruning, fertilizer application, irrigation, timing of harvest) (Bramley and Hamilton, 2004; Bramley, 2005).

Among the benefits of precision viticulture reduction of fertilizer costs, reduction of pesticide application costs, minimization of environmental pollution, increase of product yield, more accurate information management due to more efficient information production, operating records required for sales and after sales production periods.

Precision viticulture is based on the premise that high in-field variability for factors that affect vine growth and grape ripening warrants intensive management customized according to local conditions. Precision viticulture depends on new and emerging technologies such as global positioning systems (GPS), meteorological and other environmental sensors, satellite and airborne remote sensing, and geographic information systems (GIS) to assess and respond to variability (Matese and Di Gennaro, 2015).

Several authors have studied precision viticulture in different countries (Bramley et al., 2000; Bramley et al., 2003; Bramley, 2001; Bramley and Williams, 2001; Bramley and Lamb, 2003; Bramley and Hamilton, 2004, 2007; Taylor, 2004; Tisseyre et al., 2001; Arno et al., 2005; Arno, 2008; Penn, 1999; Carothers, 2000; Aho, 2002; Matese and Di Gennaro, 2015).

Vineyards are characterized by a high heterogeneity due to structural factors such as the morphological characteristics, and other dynamics such as cropping practices and seasonal weather (Bramley, 2003).

This variability causes different vine physiological response, with direct consequences on grape quality (Smart, 1985). Vineyards therefore require a specific agronomic management to satisfy the real needs of the crop, in relation to the spatial variability within the vineyard (Proffit et al., 2006). The introduction of new technologies for supporting vineyard management allows the efficiency and quality of production to be improved and, at the same time, reduces the environmental impact.

This paper presents a review of applications used in precision viticulture to production of high quality grapes.

Precision Viticulture Applications

Precision viticulture is still relatively new in that yield monitoring technology for wine grapes has only been commercially available in Australia since the 1000 vintage, and there is still only one brand of grape yield monitor on the market (although at least three others are currently under development). Nevertheless, this technology, along with other tools such as different global positioning systems (dGPS) and geographical information systems (GIS), promotes the capacity for grape and wine producers to acquire detailed geo-referenced information about vineyard performance and to start using this to tailor production of both grapes and wine according to expectations of vineyard performance, and desired goals in terms of both yield, quality and the environment (Figure 2) (Bramley and Proffitt, 1999 and 2000).

Viticulture precision process (Figure 1) begins with yield mapping and the acquisition of complementary information followed by interpretation and evaluation of the information leading to implementation of targeted management. This is followed by further observation. The process of data acquisition and use is therefore continuous, and improvements to management, incremental. Over time, data collected during the observation stage take on a predictive value (Bramley, 2001; Arno et al., 2017).

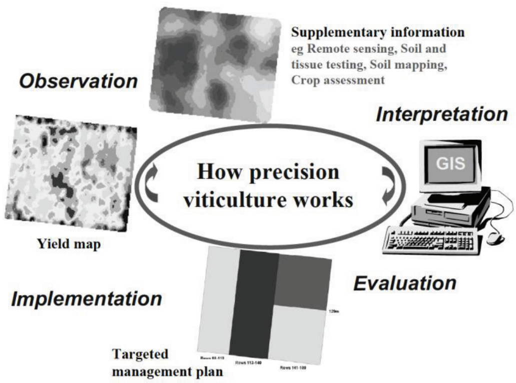


Figure 1. The process of precision viticulture (Bramley, 2001)

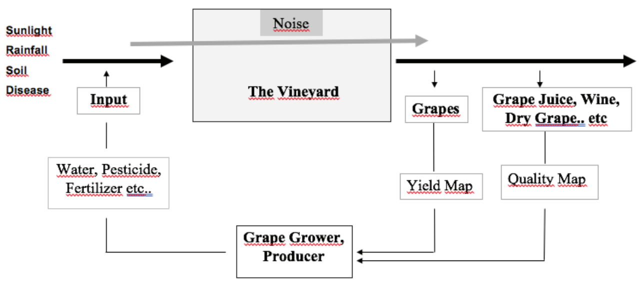


Figure 2. Viticulture input and output process

Nowadays different precision viticulture applications have helped grape growers to produce high quality grapes (Goldammer, 2015).

Terroir Management

Precision agriculture suitability to improve vineyard terroir management (Bouma, 2015). The tools of Precision Viticulture enable both growers, winemakers and researchers to see that terroir may vary within vineyards. Indeed, vineyards producing wines that are deemed characteristic of a region, may in fact be capable of producing contrasting wines from different areas within the same management units (Bramley and Hamilton, 2007).

According to Urretavizcaya et al. (2017) the early definition of within vineyard zones combining NDVI, ECa and BN data was successful, since the zones delineated allowed a differentiation of grape batches with different characteristics at harvest. Interestingly, the inclusion of a variable related to sink size (in this case the number of bunches per plant) provided the most efficient classification, which makes its consideration highly advisable for any PV work aimed at zone delineation for grape quality estimation.

Canopy Management

Canopy and vigor monitoring is the area of greatest adoption by the growers and the wineries for several reasons. It is possible to get timely, high-resolution information during the growing period, which may be relevant for canopy management, fertilization, and irrigation.

Arno et al. (2017) studied mapping the leaf area index (LAI) by using mobile terrestrial laser scanners (MTLS) is of significance for viticulture. Three different row length sections of 0.5, 1, and 2 m have been tested. Data analysis has shown that models required to estimate LAI differ significantly depending on the scanned length of the row; the model required to estimate LAI for short sections (0.5 m) is different from that required for longer sections (1 and 2 m).

According to Luo et al. (2016), grapes are likely to have collisions and be damaged by manipulations when harvesting grape clusters. For this reason, to conduct an undamaged robotic harvesting, they attempted locating the spatial coordinates of the cutting points on a peduncle of grape clusters for the end-effector and determining the bounding volume of the grape clusters for the motion planner of the manipulator using binocular stereo vision. As a result of the study, they found that cutting point detection success rate was approximately 87% and this method that it could be used on harvesting robots.

According to Berenstein et al. (2010) while much of modern agriculture is based on mass mechanized production, advances in sensing and manipulation technologies may facilitate precision autonomous operations that could improve crop yield and quality while saving energy, reducing manpower, and being environmentally friendly. They focused on autonomous spraying in vineyards and presented four machine vision algorithms that facilitate selective spraying. Researchers tested all image-processing algorithms on data from movies acquired in vineyards during the growing season. Results showed that 90% accuracy of grape cluster detection leading to 30% reduction in the use of pesticides (Gatti~~,~~ et al., 2009; Goldammer, 2015).

Tang et al. (2016) conducted a study on non-productive vine canopy estimation through proximal and remote sensing. They asserted that non-productive canopy detection in a vinicultural block is a key factor in reducing the drain on infrastructure and improving management practices and current methods are significant in cost, biased, and do not provide information on location of non-productive canopy. Researchers announced that results indicate the success of semi-supervised method in providing a useful measure of non-productive canopy at the phenological stage of veraison; laying the groundwork for improved methods in this area. They also stated that these methods provide practical outputs that lay the foundations for improving management decisions in an automatic and low-cost manner at different times in the season.

Reis et al. (2012) states that one of the most demanding tasks in wine making is harvesting, even for humans, the environment makes grape detection difficult, especially when the grapes and leaves have a similar color, which is generally the case for white grapes. In this reason, they proposed a system for the detection and location, in the natural environment, of bunches of grapes in color images. In this study, they stated that system is able to distinguish between white and red grapes, and at the same time, it calculates the location of the bunch stem. They also reported that system achieved 97% and 91% correct classifications for red and white grapes, respectively.

Escola et al. (2013) developed and tested an orchard sprayer prototype that running a variable-rate algorithm to adapt the volume application rate to the canopy volume in orchards on a real-time. They divided prototype was into three parts: the canopy characterization system (using a LiDAR sensor), the controller executing a variable-rate algorithm, and the actuators. As a result, they observed a strong relationship between the intended and the sprayed flow rates (R^2 = 0.935) and between the canopy cross-sectional areas and the sprayed flow rates (R^2 = 0.926). In addition, they state that when spraying in variable-rate mode, the prototype achieved significantly closer application coefficient values to the objective than those obtained in conventional spraying application mode.

Gil et al. (2013) announced that the structural characteristics of the canopy are a key consideration for improving the efficiency of the spray application process for tree crops. However, they state that obtaining accurate data in an easy, practical, and efficient way is an important problem to be solved. Researchers developed and tested a sprayer prototype for the suspension plant for this purpose. They electronically measured variations in canopy width along the row crop using several ultrasonic sensors placed on the sprayer and used to modify the emitted flow rate from the nozzles in real time; the objective during this process is to maintain the sprayed volume per unit canopy volume. As a result, they estimated that 21.9% less pesticides could be used compared to traditional pesticide applications. In addition, they announced that this result is in accordance with the results of similar research on automated spraying systems.

Llorens et al. (2010) compared two different spray application methods during different crop stages of three vine varieties. A conventional spray application with a constant volume rate per unit ground area was compared with a variable rate application method designed to compensate electronically for measured variations in canopy dimensions. An air-blast sprayer with individual multi-nozzle spouts was fitted with three ultrasonic sensors and three electro valves on one side, in order to modify the emitted flow rate of the nozzles according to the variability of canopy dimensions in real time. As a result, they obtained the better leaf deposits and 58% saving in application volume with variable rate method.

Crop Load Monitoring

Crop load management in vineyards is important for the consistent production of both quality fruit and mature wood. “Crop load” is the ratio of exposed leaf area to fresh fruit weight. Too much leaf area promotes shading and reduces fruit quality and sometimes bud fruitfulness. Too little leaf area per unit of fruit delays ripening and reduces vine size. Measures of crop load are useful to growers in evaluating success of vineyard management practices. The Ravaz index which uses the ratio of yield to pruning weight to estimate crop load is one common metric (Figure 3).

Research into PV is still in its infancy, and to date relatively little has been published in this field. Current and future research into PA (PV) have many of different priorities: environmental economics, production quality assessment methods and new technologies for crop monitoring (Arno et al., 2009).

In the context of precision viticulture, remote sensing in the optical domain offers a potential way to map crop structure characteristics, such as vegetation cover fraction, row orientation or leaf area index, that are later used in decision support tools. Weiss and Baret (2017) studied to Using 3D Point Clouds Derived from UAV RGB Imagery to Describe Vineyard 3D Macro-Structure.

Figure 3. (A) Spectron. (B) Multiplex hand device sensors for grape quality proximal monitoring, which allows quality maps to be realized (Matese and Di Gennaro, 2015)

Berry Quality Management

The NDVI image is an excellent tool to design quality, sampling zones based upon the NDVI classifications.
Source to sink size ratio, i.e.: the relative abundance of photosynthetically active organs (leaves) with regards to photosynthate demanding organs (mainly bunches), is widely known to be one of the main drivers of grape oenological quality. However, due to the difficulty of remote sink size estimation, Precision Viticulture (PV) has been mainly based on within-field zone delineation using vegetation indices. This approach has given only moderately satisfactory results for discriminating zones with differential quality. Urretavizcaya et al. (2017) investigate an approach to delineate within-vineyard quality zones that includes an estimator of sink size in the data-set. Zone delineation was performed using Normalized Difference Vegetation Index (NDVI), soil apparent electrical conductivity (ECa) and bunch number (BN) data.
Irrespective of the seasonal factors which affect the mean concentration of berry rotundone, variation in the land (soil, topography) underlying the vineyard is a consistent driver of within-vineyard variation in this important grape-derived flavour and aroma compound (Bramley et al., 2017).

Harvest Management

The proper ripening of grapes is the key to obtain a high-quality wine and another grape product. Ripening is a temporal process that is influenced, in addition to uncontrollable climate factors, by the spatial distribution of the vineyard and planted variety. It is a complex process that cannot be characterized by a single parameter; rather, it is a modification of the profile of the compounds of the grape. Melendez et al. (2015) analyzed the joint evolution of twelve physicochemical parameters determined in red grapes from four different varieties, in sixteen representatives (in both geographical and edaphic point of view) plots belonging to the Qualified Designation of Origin (DOC) Rioja. Samples were collected in September 2009 during four consecutive weeks prior to harvest.

Disease Management

Disease from insects, pathogens, and other infectious organisms can become a serious problem. In some cases, disease development on grapevines occur rapidly and results in entire vineyards incurring injury to various degrees. For example, grapevines are susceptible to powdery mildew infection early in the growing season. Patricia et al. (2009) studied to field monitoring for grapevine leafroll virus and mealybug in pacific northwest vineyards.
Oberti et al. (2014) conducted a study on the automatic detection of powdery mildew on grapevine leaves by image analysis. They announced that powdery mildew is a major fungal disease for grapevine (*Vitis vinifera* L.) as well as for other important specialty crops, causing severe damage, including yield loss and depreciation of wine or produce quality. According to researchers proximal optical sensing is a major candidate for becoming the preferred technique for identification of foci for powdery mildew in grapevine and other specialty crops, but detection sensitivity of symptoms in the early-middle stage can yield largely limited results due to the combination of small dimensions, low density, and spatial arrangement of thin fungal structures. They processed multi-spectral images from different angles of vine leaves under laboratory conditions. As a result, researchers found that detection sensitivity generally increases as the view angle is increased, with a peak value obtained for images acquired at 60°.
Oberti et al. (2016) developed an agricultural robot equipped with a new precision-spraying end-effector with an integrated disease-sensing system based on R-G-NIR multispectral imaging. Researchers tested the robotic system

on four different replicates of grapevine canopy plots prepared in a greenhouse setup by aligning potted plants exhibiting different levels of disease. They announced that the results indicated that the robot could automatically detect and spray from 85% to 100% of the diseased area within the canopy and to reduce the pesticide use from 65% to 85% when compared to a conventional homogeneous spraying of the canopy.

Water Management

With water becoming a more scarce and managed commodity, better management is required. Most vineyard blocks do not have the same water requirements due to differences in soil type and topography within the same vineyard. Irrigation systems have been developed that can apply the correct amount of water where it is needed.

Vineyard water status is a key aspect to reach a control about yield and quality parameters and is linked to irrigation system management. Stem and leaf water potential, in several day times, was used for monitoring, controlling and managing irrigation with good correlations with soil and plant water status and with the vegetation index (Cancela et al., 2017).

Thermal imaging can become a readily usable tool for crop agricultural water management, since it allows a quick determination of canopy surface temperature that, as linked to transpiration, can give an idea of crop water status. In the last years, the resolution of thermal imaging systems has increased and its weight decreased, fostering their implementation on Unmanned Aerial Vehicles (UAV) for civil and agricultural engineering purposes. This approach would overcome most of the limitations of on site thermal imaging, allowing mapping plant water status at either field or farm scale, taking thus into account the naturally existing or artificially induced variability at those scales. Santesteban et al. (2017) studied to evaluate to which extent high-resolution thermal imaging allows evaluating the instantaneous and seasonal variability of water status within a vineyard. The information provided by thermal images proved to be relevant at a seasonal scale as well, although it did not match seasonal trends in water status but mimicked other physiological processes occurring during ripening. Therefore, if a picture of variations in water status is required, it would be necessary to acquire thermal images at several dates along the summer.

Cancela et al. (2017) studied to test the discrimination of homogenized areas in traditional Galician vineyards of *Vitis vinifera* (L) cv. Albarifio, using a vegetation index and soil electrical conductivity and their relations with plant and soil measures (stem water potential and soil water content) and productivity and quality parameters.

Environmental Monitoring

Manually monitoring environmental parameters (e.g., humidity, temperature, soil moisture etc.) in the vineyard is not only time consuming but difficult to respond to in a timely manner when conditions change rapidly over space and time. Wireless sensor networks (WSNs), have been found to be suitable for collecting real time data for different parameters pertaining to weather, crop, and soil in developing solutions for vinicultural processes related to growing grapes. The development of wireless sensor applications in viticulture has made it possible to increase efficiency, productivity, and profitability of vineyard operations (Goldammer, 2015).

Wireless Sensor Networks

Wireless Sensor Networks (WSNs) have existed for many years and had assimilated many interesting innovations. Advances in electronics, radio transceivers, processes of IC manufacturing and development of algorithms for operation of such networks now enable creating energy-efficient devices that provide practical levels of performance and a sufficient number of features (Dziadak et al., 2016).

Wireless sensor networks deployed in vineyards is used for monitoring site conditions such as temperature, wind speed, wind direction, rainfall, solar-radiation, relative humidity, soil-moisture, soil-temperature, sap flow, and leaf wetness, for management decision making purposes. For example, Wireless sensor networks is used in the following applications:

A wireless sensor network for precision viticulture (Figure 4): The NAV (Network Avanzato per il Vigneto – Advanced Vineyard Network) system is a wireless sensor network designed and developed with the aim of remote real-time monitoring and collecting of micrometeorological parameters in a vineyard. The system includes a base agrometeorological station (Master Unit) and a series of peripheral wireless nodes (Slave Units) located in the vineyard. The Master Unit is a typical single point monitoring station placed outside the vineyard in a representative site to collect agrometeorological data. It utilizes a wireless technology for data communication and transmission with the Slave Units and remote central server (Matese et al., 2009).

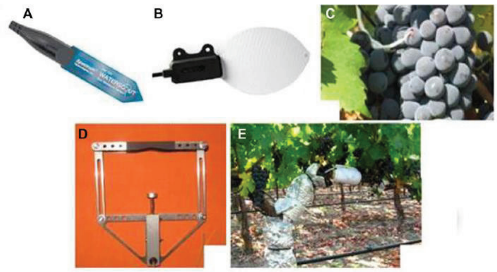


Figure 4. Some sensors employed in wireless sensor networks for proximal sensing in vineyards. (A) Soil moisture (Spectrum Technologies Aurora, IL, USA). (B) Leaf wetness (Decagon Devices Inc. Pullman, WA, USA). (C) Grape temperature. (D) Dendrometer (GMR Strumenti SAS Scandicci, Italy). (E) Sap Flow (Fruition Sciences Inc., Montpellier, France) (Matese and Di Gennaro, 2015).

Normalized Difference Vegetation Index

Agricultural remote sensing products are frequently based on so-called spectral vegetation indices (SVIs), formed as various combinations of visible and near-infrared (NIR) spectral channels of digital imagery. SVIs are radiometric variables that are useful for mapping relative variations in canopy density. One common SVI is the normalised difference vegetation index (NDVI), formulated as (NIR-red)/(NIR+red). Many commercial wine grape growers in coastal California are now using NDVI imagery, generally acquired at maximum foliar expansion, to delineate management zones, identify problems, and re-develop properties. Agricultural remote sensing products are frequently based on so-called spectral vegetation indices (SVIs), formed as various combinations of visible and near-infrared (NIR) spectral channels of digital imagery. SVIs are radiometric variables that are useful for mapping relative variations in canopy density. One common SVI is the normalised difference vegetation index (NDVI), formulated as (NIR-red)/ (NIR+red). Many commercial wine grape growers in coastal California are now using NDVI imagery, generally acquired at maximum foliar expansion, to delineate management zones, identify problems, and re-develop properties (Johnson, 2003).

By measuring the health and vigor of vegetation, NDVI can help vineyard managers fine-tune irrigation patterns. NDVI is directly related to the amount of photosynthetically active radiation that a plant may absorb (Kavak et al., 2014).

Soil Mapping

Soil electrical conductivity (EC) has been widely used to interpret soil spatial variability. Initially used to assess soil salinity, the use of EC in soil studies has expanded to include: mapping soil types; characterizing soil water content and flow patterns; assessing variations in soil texture, compaction, organic matter content, and pH; and determining the depth to subsurface horizons, stratigraphic layers or bedrock, among other uses. Variation of conductivity across soil types is the one of the main advantages of using this technology.

Weed Control

Typical vineyards may be infested by up to 20 weed species, of which three or four are dominant in terms of number of plants and land area covered. The distribution of weed species across a vineyard is “patchy” in nature. Some areas will be densely populated by weed while others will have few or no weeds. Densely populated patches often occur along vineyard edges, but may be found anywhere in the vineyard where the environment and management have favored the establishment and survival of weeds. The composition of weed species varies across a vineyard, and different patches may be dominated by different species. In addition to weed density varying spatially in a vineyard, it also may vary temporally and can be strongly influenced by weather (Goldammer, 2015).

Yield Monitoring

Grape yield maps are of fundamental importance for the development of PV (Arno et al., 2009). Yield monitoring refers to the "on-the-go" collection of both yield and positional data by the yield monitor and DGPS as the harvester travels along. The output in the form of yield maps allows growers and wine producers the ability to identify areas of different crop yield, and in some cases, different fruit quality attributes, within individual vineyard blocks. Yield maps do not require ground trothing since they represent actual as opposed to surrogate measures. Ground trothing is the process of gathering data in the vineyard that either complements or disputes remote sensing data collected by aerial photography or satellite (Gatti et al., 2009).
Kicherer et al.(2017) studied automatic image-based determination of pruning mass as a determinant for yield potential in grapevine management and breeding. Researchers calculated the mass of dormant pruning wood with the assistance of an automated image-based method for estimating the pixel area of dormant pruning wood. The evaluation of digital images in combination with depth map calculation and image segmentation is a new and non-invasive tool for objective data acquisition.
According to Aquino et al. (2015) one of the main challenges being faced by the scientific community in viticulture is early yield prediction. They have announced that flowering as well as fruit set assessment is of special interest since these two physiological processes highly influence grapevine yield. In addition, reported that an accurate fruit set evaluation can only be performed by means of flower counting. For this purpose, they presented a new methodology for segmenting inflorescence grapevine flowers in digital images. Thus, they found that values for Precision and Recall were 83.38% and 85.01%, respectively.

CONCLUSIONS

Precision viticulture is very new technology in Turkey. Hoverer, recently, precision viticulture has been received much attention in vineyard in the developed country. Different precision viticulture applications have been using and helped grape growers to produce high quality grapes. Precision viticulture depends on new and emerging technologies such as global positioning systems (GPS), meteorological and other environmental sensors, satellite and airborne remote sensing, and geographic information systems (GIS) to assess and respond to variability. It can be possible that take under control such as soil fertility, fertilizer application norm, disease, water, weed, harvesting, and environmental management by precision viticulture systems in vineyard. So, to reduce inputs such as fertilizer, water, pesticides and to increase yield and quality of grape berries, we must to increase precision technologies in our vineyards.

REFERENCES

Aho J.E., 2002. NASA providing new perspectives on vineyard management. Vineyard and Winery Management. 28(4):74–77.

Aquino A., Millan, B., Gutiérrez, S., Tardáguila, J., 2015. Grapevine flower estimation by applying artificial vision techniques on images with uncontrolled scene and multi-model analysis. Computers and Electronics in Agriculture, Vol. 119, 92-104.

Arnó J., 2008. Variabilidad Intraparcelaria en Viña y uso de Sensores Láser en Viticultura de Precisión [doctoral thesis]. [Laser sensor in Precision Viticulture to describe intra-field variability in the vineyard] Lleida: University of Lleida, Spanish.

Arnó J., Bordes X., Ribes-Dasi M., Blanco R., Rosell J.R., 2005. Esteve J. Obtaining grape yield maps and analysis of within-field variability in Raimat (Spain). Proceedings of the 5th European Conference on Precision Agriculture June 8–11, Uppsala, Sweden. 899–906.

Arno J., Escola A., Rosell-Polo J.R., 2017. Setting the optimal length to be scanned in rows of vines by using mobile terrestrial laser scanners.

Arnó J., Martinez Casasnovas J.A., Ribes Dasi M., Rosell J.R., 2009. Precision Viticulture. Research topics, challenges and opportunities in site-specific vineyard management. Spanish Journal of Agricultural Research 7(4):779-790.

Berenstein R., Ben Shahar O., Shapiro A., Edan Y., 2010. Grape clusters and foliage detection algorithms for autonomous selective vineyard sprayer. Intelligent Service Robotics, 3:233–243.

Bouma I., 2015. Interactive comment on Precision agriculture suitability to improve vineyard terroir management by J.M. Terron et al. Soil Discuss, 1, C447-C451.

Bramley R, Pearse B, Chamberlain P., 2003. Being profitable precisely – a case study of precision viticulture from Margaret River. Australian and New Zealand Grapegrower and Winemaker, 473a:84–87.

Bramley R., 2003. Smarter thinking on soil survey. Australian and New Zealand Wine Industry Journal. 18(3):88–94.

Bramley R.G.V., 2001. Progress in the development of precision viticulture – variation in yield, quality and soil properties in contrasting Australian vineyards. In: Currie LD, Loganathan P, editors. Precision Tools for Improving Land Management: Proceedings of the Workshop held by the Fertilizer and Lime Research Centre in Conjunction with the NZ Centre for Precision Agriculture at Massey University, Palmerston North, New Zealand, 14–15 February, 25–43.

Bramley R.G.V., 2005. Understanding variability in winegrape production systems. 2. Within vineyard variation in quality over several vintages. Australian Journal of Grape and Wine Research 11: 33-42.

Bramley R.G.V., Hamilton R.P., 2004. Understanding variability in winegrape production systems. 1. Within vineyard variation in yield over several vintages. Australian Journal of Grape and Wine Research 10: 32-45.

Bramley R.G.V., Hamilton R.P., 2007. Terroir and Precision Viticulture: are they compatible. J. Int. Sci. Vigne Vin, 41(1):1-8.

Bramley R.G.V., Lamb D.W., 2003. Making sense of vineyard variability in Australia. Proceedings IX Congreso Latinoamericano de Viticultura y Enología. November 24–28, Santiago, Chile. 35–54.

Bramley R.G.V., Proffitt A.P.B., 1999. Managing variability in viticultural production. The Australian Grape grower and winemaker, 427.

Bramley R.G.V., Proffitt A.P.B., Corner R.J., Evans T.D., 2000. Variation in grape yield and soil depth in two contrasting Australian vineyards. Australian and New Zealand Second Joint Soils Conference; December 3–8, Lincoln, New Zealand. 29–30.

Bramley R.G.V., Siebert T.E., Herderich M.J., Krstic M.P., 2017. Patterns of within-vineyard spatial variation in the 'pepper' compound rotundone are temporally stable from year to year. Australian Journal of Grape and Wine Research, 23(1):42-47.

Bramley R.G.V., Williams S.K., 2001. A protocol for winegrape yield maps. Proceedings of the 3rd European Conference on Precision Agriculture. June 18–21, Montpellier, France, 773–778.

Cancela, J.J., Fandino M., Rey B.J., Dafonte J., Gonzalez X.P., 2017. Discrimination of irrigation water management effects in pergola trellis system vineyards using a vegetation and soil index. Agricultural Water Management, 183:70-77.

Carothers J., 2000. Imagery technology meets vineyard management. Practical Winery and Vineyard, 21(1):54–62.

Dziadak B., Makowski L., Michalski A., 2016. Survey of Energy Harvesting systems for Wireless sensor networks in Environmental Monitoring. Metrology and Measurement Systems. 23(4):495-512.

Escola A., Rosell-Polo J.R., Planas S., Gil E., Pomar J., Camp F., Llorens J., Solanelles F., 2013, Variable Rate Sprayer. Part 1 – Orchard Prototype: Design, Implementation and Validation. Computers and Electronics in Agriculture 95, 122-135.

Gatti M., Dosso P, Maurino M., Merli M.C., Bernizzoni F., Pirez F.J. Plate B., Bertuzzi G.C., Poni S., 2009. MECS-VINE (R): A New Proximal Sensor for Segmented Mapping of Vigor and Yield Parameters on Vineyard Rows. Sensors, 16(12):1-21.

Gil E., Llrens J., Liop J., Fabregas X., Escola A., Rosell-Pol J.R., 2013. Variable Rate Sprayer. Part 2 – Vineyard Prototype: Design, Implementation and Validation. Computers and Electronics in Agriculture 95:136-150.

Goldammer T., 2015. Grape Grower's Handbook. A Guide to Viticulture for Wine Production. Apex Publishers. 728.

Johnson L.F., 2003. Temporal stability of an NDVI-LAI relationship in a Napa Valley vineyard. Australian Journal of Grape and Wine Research. 9(2):96-101.

Kavak M.T., Karadogan S., Ozdemir G., 2014. Investigating Vineyard Areas of Egil County of Diyarbakır Using Remote Sensing and GIS Techniques. International Mesopotamia Agriculture Congress, 22-25 September, 307-316, Diyarbakır, Turkey.

Kicherer A., Klodt M., Sharifzadeh S., Cremers D., Toepfer R., Herzog K., 2017. Automatic image-based determination of pruning mass as a determinant for yield potential in grapevine management and breeding. Australian Journal of Grape and Wine Research. 23(1):120-124.

Llorens J., Gil E., Llop J., Escola A., 2010. Variable rate dosing in precision viticulture: Use of electronic devices to improve application efficiency. Crop Protection, 29, 239-248.

Luo L., Tang Y., Zou X., Ye M., Li G., 2016. Vision-based extraction of spatial information in grape clusters for harvesting robots. Biosystems Engineering, Vol. 151, 90-104.

Matese A., Di Gennaro S.F., 2015. Technology in precision viticulture: a state of the art review. International Journal of Wine Research. 7:69-81.

Matese A., Di Gennaro S.F., Zaldei A., Genesio L., Vaccari F.P., 2009. A wireless sensor network for precision viticulture: The NAV system. Computers and Electronics in Agriculture 69:51-58.

Melendez E., Sarabia L.A., Ortiz M.C., 2015. Parallel factor analysis for monitoring data from a grape harvest in Qualified Designation of Origin Rioja including spatial and temporal variability. Chemometrics and Intelligent Laboratory Systems. 146:347-353.

Oberti R., Marchi M., Tirelli P., Calcante A., Iriti M., Borghese A.N., 2014. Automatic detection of powdery mildew on grapevine leaves by image analysis: Optimal view-angle range to increase the

sensitivity. Computers and Electronics in Agriculture, Vol. 104, 1-8.
Oberti R., Marchi M., Tirelli P., Calcante, A., Iriti, M., Tona, E., Hočevar, M., Baur, J., Pfaff, J., Schütz, C., Ulbrich, H., 2016. Selective spraying of grapevines for disease control using a modular agricultural robot. Biosystems Engineering, Vol. 146, 203-215.
Penn C., 1999. Grape growers gravitating toward space age technologies. In Wine Business, Monthly, Wine Communications Group, Sonoma, CA, USA.
Proffit T, Bramley R, Lamb D, Winter E., 2006. Precision Viticulture – A New Era in Vineyard Management and Wine Production. Winetitles Pty Ltd., Ashford, South Australia; 1–90.
Proffitt, T., Bramley R., Lamb D., Winter, E., 2006. Precision Viticulture: A New Era in Vineyard Management and Wine Production. WineTitles, Adelaide. ISBN 978-0-9756850-4-4.
Reis M.J.C.S., Morais R., Peres E., Pereira C., Contente O., Soares S., Valente A., Baptista J., Ferreira, P.J.S.G., Bulas Cruz J., 2012. Automatic detection of bunches of grapes in natural environment from color images. Journal of Applied Logic, Vol. 10(4):285-290.
Santesteban L.G., Di Gennaro S.F., Herrero-Langreo A., Miranda C., Royo J.B., Matese A., 2017. High-resolution UAV-based thermal imaging to estimate the instantaneous and seasonal variability of plant water status within a vineyard. Agricultural Water Management. 183:49-59.
Skinkis P.A., Dreves A.J., Walton V.M., Martin R.R., 2009. Field Monitoring for Grapevine Leafroll Virus and Mealybug in Pacific Northwest Vineyards. Oregon State University ExtensionService. EM 8985.
Smart R.E., 1985. Principles of grapevine canopy management microclimate manipulation with implications for yield and quality. American Journal of Enology and Viticulture. 1985;36(3):230–239.
Tang J., Woods M., Cossell S., Liu S., Whitty M., 2016. Non-Productive Vine Canopy Estimation through Proximal and Remote Sensing IFAC-PapersOnLine, Vol.49(16):398-403.
Taylor J.A., 2004. Digital Terroirs and Precision Viticulture: Investigations into the Application of Information Technology in Australian Vineyards [doctoral thesis]. Sydney: University of Sydney.
Tisseyre B., Mazzoni C., Ardoin N., Clipet C., 2001. Yield and harvest quality measurement in precision viticulture – application for a selective vintage. Proceedings of the 3rd European Conference on Precision Agriculture. June 18–21, Montpellier, France. 133–138.
Urretavizcaya I, Royo J.B., Miranda C., Tisseyre B., Guillaume S., Santesteban L.G., 2017. Precision Agriculture. 18(2):133-144.
Weiss M., Baret F., 2017. Using 3D Point Clouds Derived from UAV RGB Imagery to Describe Vineyard 3D Macro - Structure. Remote Sensing, 9(2): 111.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT OF BERRY SKIN, PULP AND SEED FRACTIONS OF ÖKÜZGÖZÜ AND BOĞAZKERE GRAPE CULTIVARS

Gültekin ÖZDEMIR[1], Mihdiye PİRİNÇÇİOĞLU[2], Göksel KIZIL[2], Murat KIZIL[2]

[1]Dicle University, Faculty of Agriculture, Department of Horticulture, Diyarbakir, Turkey
[2]Dicle University, Faculty of Science, Department of Chemistry, Diyarbakir, Turkey

Corresponding author email: gozdemir@dicle.edu.tr

Abstract

*Grape cultivars (*Vitis vinifera *L.) are believed to have health benefits due to their antioxidant activityand phenolic content. Thus, scientists have conducted research to explore their positive effects on many human diseases. The aim of this study was to determinetotal phenolic and flavonoid contents of berry pulp, seed and skin of Öküzgözü and Boğazkere red wine grape cultivars grown in Turkey. In conclusion, it was found that total phenolic (µg GAE/mg) and flavonoid content in Öküzgözü and Boğazkere grape cultivars showed importantdifferences according to the berry skin, pulp, seed and research years. The highest phenolic content was found in Öküzgözü berry pulp 803.00 µg GAE/mg in 2012 year. When the flavonoid amounts are compared, it has been determined that the total flavonoid amount varied from 5.08 µg QUE/mg to 111.55 µg QUE/mg. The highest flavonoid content was found in the Öküzgözü grape berry skin in 2011 year (111.55 µg QUE/mg). This study showed that these grapes are a potential source of phenolic and flavonoid compounds. It can be concluded that selected grape varieties and their parts can be considered a good source of phenolics.*

Key words*: Grape, Öküzgözü, Boğazkere, Berry, Phenolic, Flavonoid.*

INTRODUCTION

Turkey is one of the top producers of grape. It has 467,093 ha of vineyards and a production of 4.1 million tons. Over 74 million tons of grapes are grown worldwide on more than 7.1 million ha. Turkey ranks fifth in terms of growing area, after Spain, France, China, and Italy, and ranks sixth in production after China, Italy, USA, Spain and France (Anonymous, 2014).

In Turkey, grapes have been mainly grown as table grapes (52%), for raisins (38%), and for fruit juice and wine (10%), with around 80 standard cultivars grafted onto mainly six standard rootstocks, in nine viticultural regions. Turkey has about 7% of the world's area of vineyards, and produces 6.4% of the world's grape production. In addition, productivity in Turkey has improved by about 40% in the last 15 years, from 6654 kg ha^{-1} in 1998 to 9249 kg ha^{-1} in 2012 (Soylemeoglu et al., 2016).

Turkey is the one of the gene center of grapevines, for this reason it possesses over 1200 grape varieties. Nearly all grape varieties grown in Turkey are european-type grapes (*Vitis vinifera* L.).

The types and concentration of the phenolic compounds depend on the grape variety, ripening, climatic conditions, wine making practices (the use of enzymes, maceration conditions, and fermentation temperature), and ageing (Kelebek et al., 2007).

Among the types of grapes, especially red grapes and grape juice, the major phenolic compounds found in red wine are called as flavonoids, anthocyanins and flavonols (Rice-Evans et al., 1996; Singleton, 1982; Palomino et al., 2000). It is reported that these substances that are important in terms of human health and found in grape(Morris and Cawthon, 1982; Bravdo et al., 1985; Matthews and Anderson, 1988; Iland, 1989; Nadal and Arola, 1995; De La Hera Orts et al., 2005; Pirinccioglu et al., 2012; Ozdemir et al., 2016) vary according to the varieties of the grapes (Landrault et al., 2001), climate and soil conditions of the place

where it grows (Spayd et al., 2002; Mateus et al., 2001).
Among the climate features of the vineyard areas, the place and vector issues especially the temperature, humidity and sunlight are encountered as the important factors affecting the synthesis the of phenolic compounds.
Some authors have studied the total phenolic and flavonoid contents of different grape cultivars and ecological regions in Turkey (Deryaoglu and Canbas, 2003; Aras, 2006; Orak, 2007; Babalik et al., 2009; Baydar et al., 2009; Uluocak, 2010; Kelebek, 2009; Bayir, 2011; Cangi et al., 2011; Toprak, 2011; Kaplama, 2012; Pehlivan et al., 2015).
Öküzgözü and Boğazkere are red grape cultivars of *Vitis vinifera* L. grown in eastern Turkey, especially Elazig, Malatya, and Diyarbakir provinces. It is an important red grape variety for Turkey, which produces well-balanced and characteristics wines, with fruity notes such as strawberry, cherry, and blackberry-like odours (Cabaroglu et al., 2002; Kelebek et al., 2007)
The aim of the present study was to determine the total phenolic and flavonoid content of berry skin, pulp and seed of Öküzgözü and Boğazkere grape cultivars.

MATERIALS AND METHODS

Plant Material

This research was carried out in the Dicle University Department of Horticulture and Chemistry in 2011, 2012 and 2013 years. In the research, Öküzgözü and Boğazkere (*Vitis vinifera* L.) Turkish wine grape cultivars were used as biological material (Figures 1 and 2).
Grape varieties are grown in Elazığ (Sün Village) province.
Fresh grapes from Boğazkere and Öküzgözü cultivars were manually harvested at optimum maturity in the 2011, 2012 and 2013 vintage in Elazig province and transported to the Plant Physiology Laboratory at the Department of Horticulture, University of Dicle, located in Diyarbakir, Turkey.

Determination of total phenolic and flavonoid contents

Total phenolic and flavonoid content of the grapes obtained with different part of berries (skin, pulp, seed) (Figure 1, Figure 2) from Boğazkere and Öküzgözü grape cultivars.
Total phenolic content was determined according to Le et al. (2007); gallic acid (10–180 μg/mL) was used as standard. Samples of 40 μL of extract solution (1 mg/mL) were mixed with 200 μL Folin–Ciocalteau's phenol reagent 10% in water. After 4 min of incubation, 0.4 mL of 20% Na_2CO_3 was added. The reaction tubes were further incubated for 2 h at room temperature and the absorbance was measured at 760 nm. The concentration of total phenolic compounds in the extract was determined as μg of gallic acid equivalents per mg of extract (μg GAE/mg) (Kada et al., 2016; Ozdemir et al., 2016).
Total flavonoid content was quantified according to Bahorun et al. (1996) using quercetin (2–20 μg/mL) as standard. Briefly, samples of 1 mL of extract solution (1 mg/mL) were incubated in the presence of 1 mL of $AlCl_3$ (2%) for 10 min at room temperature. The absorbance was measured at 430 nm. Total flavonoid content was expressed as μg quercetin equivalent per mg of extract (μg QUE/mg) (Kada et al., 2016; Ozdemir et al., 2016).

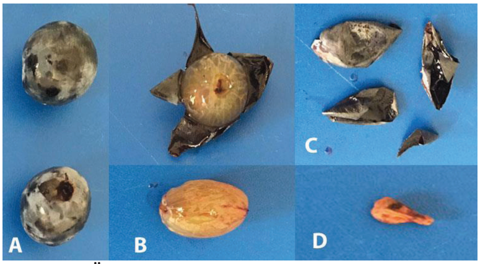


Figure 1. Öküzgözü (*Vitis vinifera* L.cv) (A) berry (B) skin, (C) pulp and (D) seed

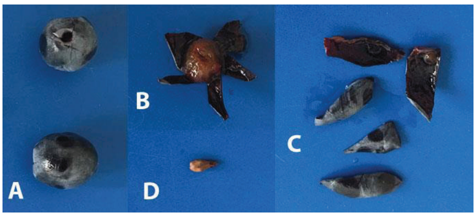


Figure 2. Boğazkere (*Vitis vinifera* L. cv) (A) berry (B) skin, (C) pulp and (D) seed

RESULTS AND DISCUSSIONS

As a result of the study, total phenolic (μg GAE/mg) content of the grape cultivars showed differences according to the berry fractions (skin, pulp and seed) and years.

Total phenolic content varied from 85.45 µg GAE/mg to 126.70 µg GAE/mg in Öküzgözü and Boğazkere grape berry skin.

The highest values in Öküzgözü berry skin were found in 2013 year (average 89.58 µg GAE/mg).

The maximum amount of phenolic content was found in Boğazkere variety in 2012 (126.70 µg GAE/mg) (Table 1).

Table 1. Total phenolic content (µg GAE/mg) in grape berry skin

	Total phenolic content			
Cultivars	2011	2012	2013	Average
Öküzgözü	85.45	81.25	102.05	89.58
Boğazkere	100.55	126.70	107.00	111.42

Total phenolic content varied from 493.70 µg GAE/mg to 766.40 µg GAE/mg in Öküzgözü and Boğazkere grape berry pulp.

The maximum amount of phenolic content was found in Öküzgözü variety in 2013 (766.40 µg GAE/mg).

The least amount was found in the Boğazkere grape variety in 2011 (493.70 µg GAE/mg). The average values in berry skin were found in 89.58 µg GAE/mg in Öküzgözü and 523.43 µg GAE/mg in Boğazkere variety (Table 2).

Table 2. Total phenolic content (µg GAE/mg) in grape berry pulp

	Total phenolic content			
Cultivars	2011	2012	2013	Average
Öküzgözü	704.40	803.00	766.40	757.93
Boğazkere	493.70	546.60	530.00	523.43

Total phenolic content varied from 157.60 µg GAE/mg to 340.40 µg GAE/mg in grapes berry seed.

The average values in berry seed were found in 182.75 µg GAE/mg in Öküzgözü and 329.45 µg GAE/mg in Boğazkere variety.

The highest amount of phenolic content was found in Boğazkere variety in 2012 (340.40 µg GAE/mg) (Table 3).

Table 3. Total phenolic content (µg GAE/mg) in grape berry seed

	Total phenolic content			
Cultivars	2011	2012	2013	Average
Öküzgözü	157.60	183.30	207.35	182.75
Boğazkere	327.70	340.40	320.25	329.45

The total flavonoid content found in the berry skin was detected to vary from 36.16 to 111.55 µg QUE/mg. It has been detected that the flavonoid content in the skin was found in Öküzgözü grape variety in 2011 year (111.55 µg QUE/mg) and the lowest one was found in Boğazkere variety in 2013 year (36.16 mg QUE/mg). The average values in berry skin were found to be: 108.44 µg QUE/mg in Öküzgözü and 48.35 µg QUE/mg in Boğazkere variety (Table 4).

Table 4. Total flavonoid content (µg QUE/mg) in grape berry skin

	Total flavonoid content			
Cultivars	2011	2012	2013	Average
Öküzgözü	111.55	107.01	106.77	108.44
Boğazkere	54.11	54.79	36.16	48.35

Total flavonoid content varied from 17.20 µg QUE/mg to 39.66 µg QUE/mg in Öküzgözü and Boğazkere grape berry pulp. The highest flavonoid content in the pulp was found to be 39.66 µg QUE/mg in Boğazkere grape variety in 2013 year. The lowest one was found to be 17.20 µg QUE/mg in Öküzgözü variety in 2011 year. The average flavonoid values in berry pulp were found to be: 17.32 µg QUE/mg in Öküzgözü and 29.65 µg QUE/mg in Boğazkere variety (Table 5).

Table 5. Total flavonoid content (µg QUE/mg) in grape berry pulp

	Total flavonoid content			
Cultivars	2011	2012	2013	Average
Öküzgözü	17.20	17.40	17.37	17.32
Boğazkere	24.54	24.76	39.66	29.65

Total flavonoid content varied from 5.08 µg QUE/mg to 11.23 µg QUE/mg in grape berry seed.
The highest flavonoid content in the seed was found to be 11.23 µg QUE/mg in Boğazkere grape variety in 2013 year.
The lowest one was found to be 5.08 µg QUE/mg in Öküzgözü variety in 2012 year. The average flavonoid values in berry seed were found to be: 5.14 µg QUE/mg in Öküzgözü and 9.88 µg QUE/mg in Boğazkere variety (Table 6).

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

	Total flavonoid content			
Cultivars	2011	2012	2013	Average
Öküzgözü	5.20	5.08	5.16	5.14
Boğazkere	8.08	10.34	11.23	9.88

It has been identified in different research that total phenolic amounts vary according to the variety and year and decreased during maturation period (Doshi et al., 2006; Navarro et al., 2008; Jin et al., 2009). Saidani Tounsia et al., (2009) examined the total phenolic amount in the methanol extract of three types of red grape seeds and found the equivalent of respectively 427.00 mg/100g, 218.00 mg/100g and 112.81 mg/100g of gallic acid for dry weights of Muscat, Shiraz and Carignan varieties. A similar study was carried out by Hogan et al. (2009) and it was examined the total phenolic content of the three types of Virginia black wine grapes in various regions of northern France and Cabernet in Virginia black wine grapes in northern France as Cabernet Franc 1, Cabernet Franc 2 and Cabernet Franc 3 and, as a result, they were identified to be equivalent of respectively 1.82 ± 0.07 mg/g, 1.47 ± 0.05 mg/g, 0.63 ± 0.02 mg/g of gallic acid.
As a result of their study, Ozden and Vardin (2009) have found that the total phenolic compound concentration of some grape varieties grown in Sanliurfa conditions such as Merlot, Chardonnay, Cabernet Sauvignon and Shiraz (*V. vinifera* L.) grape varieties vary from 1805 mg/kg to 3170 mg/kg in terms of total phytochemical properties. While the highest concentration of phenolic compounds was found in Chardonnay variety, the lowest concentration was found in Shiraz variety.
In their study, Gokturk Baydar et al., (2011) have identified grape seeds and skin extracts belonging to Cabernet Sauvignon, Kalecik Karasi and Narince grape varieties, antioxidant properties of wine and the content of phenolic compounds. Total phenolic content was determined to vary from 522.49 to 546.50 mg GAE g^{-1} in seed extracts and from 22.73 to 43.75 mg GAE g^{-1} in skin extracts and from 217.06 to 1336.21 mg L^{-1} in wine. The radical scavenging effects of the samples and reducing capacities varied depending on grape varieties, the parts of the grape and the wine type.
Kanner et al. (1994) analyzed total phenolic compound amounts by harvesting the grapes in optimal harvest ripeness and the study was conducted with seven different table (Miabell Concord, Flame Seedless, Emperor, Thomson Seedless, Red Globe and Red Malaga) and seven different wine (Calzin Petite Shiraz, Merlot, Cabernet Sauvignon, Cabernet Franc, Sauvignon Blanc and Chardonnay) grapes. They reported that phenolic compounds in wine grapes vary from 230 to 1236 mg/l and Calzin and Petit Shiraz grape varieties have the highest phenolic content.
As a result of the analysis made in grape varieties examined in Elazig conditions, the total amount of phenolic and flavonoid content in the pulp, skin and seed of the berry were found to vary greatly among varieties and research years (Tables 1, 2, 3, 5 and 6).

CONCLUSIONS

It was found that total phenolic (µg GAE/mg) and flavonoid content in Öküzgözü and Boğazkere grape cultivars showed important differences according to the berry skin, pulp, seed and years.
In this research, the highest values in terms of the amount of phenolic content have been determined in the case of the pulp 803.00 µg GAE/mg in Öküzgözügrape variety.
The highest phenolic content was noticed in Öküzgözü berry seed being207.35 µg GAE/mg in 2013 year. In the skin of in Öküzgözü variety, in 2013 phenolic content was found to be 102.05 µg GAE/mg.

When the flavonoid amounts are compared, it has been determined that the total flavonoid amount varied from 5.08 µg QUE/mg to 111.55 µg QUE/mg. The highest flavonoid content was found in the Öküzgözü grape berry skin in 2011 year (111.55 µg QUE/mg).

Among plant-derived foods, fruits and vegetables are natural sources that are rich in phenolic substances. Today, it is clear that the increase in escaping from the artificial substances will increase the significance of the natural phenolic substances. Besides the use opportunities in the fields of food, ladder and pharmacology, it is seen that understanding the mechanism of action of phenolic substances with significant effects on human health and it is important to investigate the paths to be able to use technologically (Ozdemir et al., 2016).

ACKNOWLEDGEMENTS

The authors thank Dicle University Scientific Research Project Coordinatory for its funding of this research (Project Number: 15-ZF-14).

REFERENCES

Anonymous, 2014. Food and Agriculture Organization of United Nations Crops (FAO) Database. http://www.fao.org/faostat/

Aras O., 2006. Determination of the Total Carbohydrate, Protein, Mineral Substances and Phenolic Compounds of Grape and Grape Products. Suleyman Demirel University, Graduate School of Natural and Applied Sciences M.Sc. Thesis, 59p.

Babalik Z. Cetin S. Hallac Turk F., Gokturk Baydar N., 2009. Determination of Phenolic Compounds of Cavus Grape Cultivars under Different Training Systems. 7th Symposium on Viticulture and Technologies, 5-9 October, Turkey.

Bahorun T, Gressier B, Trotin F, Brunet C, Dine T, Luyckx M, Vasseur J, Cazin M, Cazin JC, Pinkas M. 1996. Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. Arzneimittelforschung. 46:1086–1089.

Baydar G. N., Ozkan G., Yasar S., 2007. Evaluation of the Antiradical and Antioxidant Potential of Grape Extracts. Food Control, 18, 1131-1136.

Bayir A., 2011. Investigation of Phenoloic Content and Antiradical Activity of Grape, Mulberry and Myrtle. Akdeniz University, Graduate School of Natural and Applied Sciences PhD Thesis, 147p.

Bravdo B.A., Hepner Y., Loigner C., Cohen S., Tabacman H., 1985. Effect of irrigation and crop level on growth, yield, and wine quality of Cabernet Sauvignon. American Journal of Enology and Viticulture. 36: 132-139.

Cabaroglu T., Canbas A., Lepoutre J.P., Gunata Z., 2002. Free and bound volatile composition of red wines Vitis vinifere L. cv. Öküzgözü and Boğazkere grown in Turkey. America Journal of Enology and Viticulture 53:64-68.

Cangi R., Saracoglu O., Uluocak E., Kilic, D., Sen A., 2011. The Chemical Changes of Some Wine Grape Varieties During Ripening Period in Kazova (Tokat) Ecology. Igdir Univ. J. Inst. Sci. & Tech. 1(3): 9-14

De La Hera Orts M.L., Martinez-Cutillas A., Opez-Roca J.M., Gomez-Plaza E., 2005. Effect of moderate irrigation on grape composition during riperning. Spanish Journal of Agricultural Research. 3: 352-361.

Deryaoglu A., Canbas A., 2003. Physical and Chemical Changes Occured During Maturation of Okuzgozu Grape Variety Grown in Elazig Region. The Journal of Food. 28(2):131-140.

Doshi P., Adsule P., Banerjee K., 2006. Phenolic Composition and Antioxidant Activity In Grapevine Parts and Berries (Vitis vinifera L.) cv. Kishmish Chornyi (Sharad Seedless) During Maturation. International Journal of Food Science and Technology, 41 (Supplement 1), 1–9.

Gokturk Baydar N., Babalik Z., Hallac Turk F., Cetin E.S., 2011. Phenolic Composition and Antioxidant Activities of Wines and Extracts of Some Grape Varieties Grown in Turkey. Journal of Agricultural Sciences, 17(2011), 67-76.

Hogan S., Zhang L., Li J., Zoecklein B., Zhou K., 2009. Antioxidant properties and bioactive components of Norton (Vitis aestivalis). Food Science And Technology, 42, 1269-1274.

Iland P., 1989. Grape berry composition-the influence of environmental and viticultural factors, Australian Grape grower & Winemaker. 302: 13-15.

Jin Z.M., He J.J., Bi H.Q., Cui X.Y., Duan C.Q., 2009. Phenolic Compound Profiles in Berry Skins From Nine Red Wine Grape Cultivars in Northwest China. Molecules, 14(12), 4922-4935.

Kada S., Bouriche H., Senator A., Demirtaş İ., Özen T., Toptanci B.Ç., Kızıl G., Kızıl M., 2016. Protective activity of Hertia cheirifolia extracts against DNA damage, lipid peroxidation and protein oxidation. Pharmaceutical Biology, 55(1):330-337.

Kanner J., Frankel E., Granit R., German B., Kinsella J.E., 1994. Natural Antioxidants in Grape and Wines. Ibid. 42, 64-69.

Kaplama P., 2012. Antioxidant Activities Anthocyanin Profiles and some Physical and Chemical Properties of Grape Cultivars Grown in Erzincan. Ataturk University, Graduate School of Natural and Applied Sciences M.Sc. Thesis, 87p.

Kelebek H., 2009. Researches on the Phenolic Compounds Profile of Okuzgozu, Bogazkere and Kalecik Karasi Cultivars Grown in Different Regions and Their Wines. Cukurova University, Graduate School of Natural and Applied Sciences PhD Thesis, 251p.

Kelebek H.K., Canbas A., Cabaroglu T., Selli S., 2007. Improvement of antocyanin content in the cv

Öküzgözü wines by using pectolytic enzymes. Food Chemistry 105(1):334-339
Landrault N, Poucheret P, Ravel P, Gasc, Cros G, Teissedre PL., 2001. Antioxidant capacities and phenolic l evels of French wines from different varieties and vintages. J. Agr. Food Chem, 49: 3341-3348.
Le K, Chiu F, Ng K., 2007. Identification and quantification of antioxidants in Fructus lycii. Food Chem. 105:353–363.
Mateus N., Proença S., Ribeiro P., Machado J.M., De Freitas V., 2001. Grape and wine polyphenolic composition of red Vitis vinifera varieties concerning vineyard altitude, Cienc. Technol., Aliment., 3(2): pp.102-110.
Matthews M., Anderson, M., 1988. Fruit ripening in Vitis vinifera L responses to seasonal water deficits, American Journal of Enology and Viticulture, 39:313-320.
Morris J.R., Cawthon D.L., 1982. Effect of irrigation, fruit load, and potassium fertilization on yield, quality, and petiole analysis of concord (Vitis vinifera L) grapes, American Journal of Enology and Viticulture, 33: 145-148.
Nadal M., Arola L., 1995. Effects of limited irrigation on the composition of must and wine of Cabernet Sauvignon under semi-arid conditions, Vitis, 34: 151-154.
Navarro S., Leo´N M., Roca-Pe´Rez L., Boluda R., Garcı´A-Ferriz L., Pe´Rez-Bermu´Dez P., Gavidia I., 2008. Characterisation Of Bobal And Crujidera Grape Cultivars, In Comparison With Tempranillo And Cabernet Sauvignon: Evolution Of Leaf Macronutrients And Berry Composition During Grape Ripening Food Chemistry 108, 182–190.
Orak H.H., 2007. Total Antioxidant Activities, Phenolics, Anthocyanins, Polyphenoloxidase Activities of Selected Red Grape Cultivars and Their Correlations Scientia Hort. Vol. 111, Issue 3, 5 February 2007, Pages 235-241.
Ozdemir G., Beren Sogut A., Pirinccioglu M., Kizil G., Kizil M., 2016. Changes in The Phytochemical Components in Wine Grape Varieties During The Ripening Period. Scientific Papers. Series B, Horticulture. Vol. LX, 85-93.
Ozden M., Vardin H., 2009. Quality and Phytochemical Properties of some Grapevine Cultivars Grown in Sanliurfa Conditions. Harran Journal of Agriculture and Food Science 13(2): 21-27.
Palomino O., Gomez-Serranillos M.P., Slowing K., Carretero E., Villar A., 2000. Studyof polyphenols in grape berries by reversed-phase highperfor mance liquid chromatography. Journal of Chromatography, A. 870: 449-451.
Pehlivan E.C., Uzun H.İ., 2015. Effects of Cluster Thinning on Yield and Quality Characteristics in Shiraz Grape Cultivar. Yuzuncu Yil University Journal of Agricultural Sciences 25 (2):119-126.
Pirinccioglu M., Kızıl G., M Kızıl., Ozdemir G., Kanay Z., Ketani M.A., 2012. Protective effect of öküzgözü grape (Vitis vinifera L.) juice against carbon tetrachloride induced oxidative stress in rats. Journal of Food Funct., 3, 668-673.
Rice-Evans C.A., Miller N.J., Paganda G., 1996. Structure antioxidant activity relationship of flavonoids and phenolic acids, Free Radical Biology & Medicine, 20: 933-956.
Saidani Tounsia, M., Ouerghemmi, I., Wannes, W.A., 2009. Ksouri, H.Z.; Marzouk, B.; Kchouk, M.E. Industrial Crops and Products, 30, 292–296.
Singleton V.L., 1982. Grape and wine phenolics: background and prospects. In: Webb, A.D. (ed.), Proceedings of Grape Wine Centennial Symposium University of California, Davis 215-222.
Soylemezoglu G., Atak A., Boz Y., Unal A., Saglam M., 2016. Viticulture in Turkey. Chronica Horticulturae 56 (2): 27-31.
Spayd S.E., Tarara J.M., Mee D.L., 2002. Ferguson, J.C., Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries. Am. J. Enol. Vitic., 53(3): pp.171-182.
Toprak F.E., 2011. Phytochemical Characteristics in Kalecik Karasi Grape Cultivar (Vitis vinifera L.) Grown in Ankara and Nevsehir. Ankara University, Graduate School of Natural and Applied Sciences M.Sc. Thesis, 64p.
Uluocak E., 2010. The physical and chemical changes during ripening period of some wine grape varieties grown in Kazova (Tokat) Ecology. Gaziosmanpasa University, Graduate School of Natural and Applied Sciences M.Sc. Thesis, 89p.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE EFFECTS OF DROUGHT ON THE LEVEL OF ISOFORMS OF AQUAPORIN IN CV. 'HOROZKARASI' GRAPEVINE

Mehmet KOÇ[1], İbrahim Samet GÖKÇEN[1], Mehmet İlhan ODABAŞIOĞLU[2], Kenan YILDIZ[3]

[1]Aralık University, Faculty of Agriculture, Department of Horticulture, Kilis 7, Turkey
[2]Harran University, Faculty of Agriculture, Department of Horticulture, Şanlıurfa, Turkey
[3]Gaziosmanpaşa University, Faculty of Agriculture, Department of Horticulture, Tokat, Turkey

Corresponding author email: mk_mehmetkoc@outlook.com

Abstract

This study aimed to investigate the aquaporin expression of 'Horozkarası' grapevine. Therefore, own-rooted cv. 'Horozkarası' grapevines were exposed to two different irrigation treatments; well-irrigated and water-stress treatments under controlled environmental conditions in pots for six days. And then in the stressed plants lipid peroxidation (MDA), relative water content (RWC) and membrane permeability (MP) were measured in leaves. The expression patterns of different PIPs group of aquaporins (PIP2-1, PIP2-2) also were performed with root and leaf tissues. While significant decreases were observed in relative water content (RWC) and membrane permeability (MP) in stress treatment, increase was observed in lipid peroxidation (MDA) in leaves. Also, while significant decreases were observed in PIP2-1 and PIP2-2 in stress treatment in leaves, increases were observed in expressions of PIP2-1 and PIP2-2 genes in drought treatment in root.

***Key words**: Vitis, Aquaporins, water stress, MDA.*

INTRODUCTION

'Horozkarası' grapevine has significant economic importance, and is grown in Southeastern Anatolia, especially in Gaziantep and Kilis, for using in vine and dried grape production. Because of the increase in demand on fruits having high antioxidant content that is important for human health, the demand on 'Horozkarası' grapevine that is widely consumed in dried form started to increase day by day.

Its long, elliptic, and significantly large grapes make this variety to be consumed in dried form, as well as it is also consumed freshly. 'Horozkarası' grapevine grape, which is very common especially in Gaziantep and Kilis region, is grown in almost all of the cities in GAP region (Gürsöz, 1993).

This variety is also known as Kilis Karası and Antep Karası among the producers and, among 35-40 sorts of grapes grown in Gaziantep province, it comes to the forefront with its area and amount of production.

Grapevine is a cultivation plant that has high level of adaptability to inappropriate soil conditions. Except for a narrow line in eastern Black Sea region, all of the regions of Turkey are within the arid and semi-arid climate belt. Moreover, as in entire world, the reflections of global warming also increase in Turkey. Besides the global warming, especially the decrease in usable water sources, the increase in arid and semi-arid agricultural lands, and the significant increases in duration and severity of drought have significantly stressful effect on the cultivated plants being grown. As well as it decreases the yield and quality, it might also result in increase in product losses and even in death of plants. On the other hand, it is another important research topic if the actual agricultural lands, on which the agricultural activities are performed, will be suitable for agricultural production in future. Some of the studies on this subject indicate that the patterns in agricultural production might change in future due to the climate change. It is inevitable that such a climate change will directly affect the viniculture in future. Today's vineyards will be unsuitable for vinicultural activities under the effects of increasing temperature and limited water sources. Scientists make effort for developing solution suggestions for this problem; there are 2 solution suggestions. First of them is to obtain new hybrids by crossing actual varieties (*V. vinifera* L.) with those having high drought tolerance to be used in future. The second proposal is to evaluate the new microclimate regions to occur in the regions close to the poles due to the increasing temperatures. In order both to protect the actual limited water sources we have and to obtain high crop yield and quality by using relatively lower level of water use, it is necessary to determine the drought tolerance of existing grape varieties. Thus, in near future, it would be relatively easier to obtain varieties to be grown using less water and to have quality suitable for consumption and market requirements by utilizing varieties with high tolerance to drought. Even if it is attempted to increase the efficiency of water use through the technological advancements regarding the irrigation systems and to protect the water sources, these precautions are limited, short-dated, and very expensive. For this reason, it is very important to select the grapevine varieties having high drought-tolerance and to examine the effect of their interaction with rootstock on the drought tolerance.

The leading one among the most important abiotic factors affecting the quality and yield in agricultural production is the drought. The grape gives physiological, biochemical, and molecular responses to the drought. The chemical signals coming from the roots play important role in the adaptation especially in first phase of water stress (Schachman, 2008). The signals are conveyed through the xylem to the leaves, and play role in arranging the water losses. Under the conditions of drought, many chemical signals are transmitted from the roots to the leaves. Some of these chemical signals, abscisic acid (ABA), pH, cytokine, malate, and precursor compounds of ethylene play role in the use of water during the first phase of drought stress (Schachtman, 2008). ABA was reported to act as stress hormone under the environmental conditions such as drought and salt stress (Peleg, 2011; Fukaki 2009). But, regarding the importance of ABA for the root signals, there are debates among the studies due to the methodological differences (Schachman, 2008). It was reported that, during the drought stress, the pH changes in xylem core play role as chemical signal (Wilkinson, 1999). This change affects ABA metabolism or directly the water status of leaves. pH change in xylem causes the close of stoma by activating the ABA that is the cell protector (Wilkinson, 1999). In studies on the chlorophyll content of drought stress in grapes, when compared to irrigated plants, the increases were reported in the total chlorophyll content of unirrigated grapes by Maroco et al. (2000), while Flexas et al. (2000) reported remarkable decrease and Chaumont et al. (1994) found no change in total chlorophyll content.

In drought stress tolerance of various varieties, a close relationship was found between the antioxidant system and the decrease in oxidative damage (Zhang and Kirkham, 1996; Jiang and Zhang, 2002; Lima et al., 2002; Ramachandra et al., 2004; Sofo et al., 2005; Sanchez-Diaz et al., 2007; Aganchich et al., 2009; Ozkur et al., 2009; Wang et al., 2009). In their study on the antioxidative mechanism in drought adaptation of two varieties grown on their roots (Sabatiano and Mavrodafni), Alexandros and Angelos (2012) reported a rapid increase in hydrogen peroxide concentration of Mavrodafni variety under droughty conditions. The researchers emphasized that, while there was no significant change in CAT activity under drought stress, there was difference in APX and SOD activities.

The transportation of water in xylem in angiosperms is known to be affected from the anatomical characteristics such as vein size, distribution and intensity, core structure, vein permeability, and topology of xylem network. Besides them, the chemical signals (ABA) significantly affect the water transportation in plants in expression of aquaporin. Lovisolo et al. (2008) emphasized that ABA has important role in transpirational control in rehydrated grapes, and reported that the xylem embolism and abscisic acid hormone increased in stressed plants. In addition, they showed that aquaporin has important role in regulation of the leaf and root hydraulics. Aquaporin is a water channel protein that exists in various physiological processes among the organisms. In grapes, aquaporins play important role in drought adaptation of plants by maintaining their ion and water balance under varying environmental conditions. In studies on different plant varieties, among the plant that have not been exposed to drought stress, PIP1;1 aquaporin gene was expressed in roots at higher levels when compared to the expression in leaves (Galmes et al., 2007; Weig et al., 1997; Jang et al., 2004). It is known that, aquaporin plays important role in adaptation of grapes to drought due to varying environmental conditions. Aquaporins play important role in arranging the hydraulics in roots (Vandeleur et al., 2009) and leaves (Pou et al., 2013) of grapevines. Aquaporins play role in continuous root-to-leaf water transportation, and they might lead to rapid and inverse changes in cell's hydraulic conductivity by arranging the water permeability of membrane (Hayes et al., 2007; Surbanovski and Grand, 2014). Aquaporins play role in arranging the water movement throughout the plasma membranes in the metabolic path between the cells and in correcting the xylem embolization (Lovisolo and Schubert, 2006). Zarrouk et al. (2015) reported that, in their study on drought in cv. Toriga Nacional grape variety, the root-shoot signals responded by increasing the root hydraulic conductivity in mid-level water stress by being encouraged by the chemicals. In addition, they emphasized that the aquaporin isoforms played role as major-sub organizer and in sensing the water stress since the first phase of water stress.

MATERIALS AND METHODS

Growing the Plants, Implementation, and Taking the Samples

This study was carried out in greenhouses within the body of Kilis 7 Aralık University's Agricultural Implementation and Research Center (TUAM) and in Agricultural Engineering Faculty Laboratory of the university. The slips used in this study were collected from the vineyards in this region. The materials collected were cut into the suitable size for rooting, and then planted into rooting cases by sinking into 2% fungicide solution. The rooted healthy plants were planted into 5 L (1:1 v:v) peat/perlite mixture. In order to endure the homogeneity, they were grown on a single body with cut branches. As of the month of August, the plants were irrigated on regular basis, and then they were divided into control and drought groups. This study was carried out in experimental pattern of fully randomized coincidence parcels, in triplicated in accordance with factorial order (10 healthy saplings planted in pots in each repeat). Drought regime was implemented for 6 days. At the end of 6^{th} day, the root and leaf samples were immediately treated with liquid nitrogen for molecular analyses, and kept at 80˚C until the analyses.

Relative Moisture Content (RMC)

The leaf samples collected a little while before the harvest were immediately weighed and the wet weight (WW) was determined. By keeping the samples in pure water for 4 hours, they were transformed into turgor, and then weighed again (TW). And, finally, the leaf samples were dried in drying cabinet at 60°C for 24 hours, and dry weight (DW) was determined (Dhanda and Sethi (1998). Using the formula below, the relative moisture content was calculated.

$$RMC\ (\%) = [(WW-DW)/(TW-DW)]x100$$

Membrane Permeability (MP)

The leaf samples taken before the harvest (0.1 g) were rinsed firstly with tap water and then with pure water. The plant samples were kept in 10 ml pure water at 40°C for 30 minutes, and EC (C1) of solution was measured (C1). EC was measured again for the sample that was kept in water bath at 100°C for 10 minutes (C2), and MSI was calculated using the formula below (Premchandra et al., 1990 and Sairam, 1994).

$$MP= [1-(C1/C2)]\ x\ 100$$

Determining the Lipid Peroxidation (MDA)

In order to show the stress effect on plant and to compare with the levels of gene expression, malondialdehyde (MDA) analysis was employed in determining the lipide peroxidation (Hodges et al., 1999). In calculating the values read on spectrophotometer and percentage MDA levels, the formulas below were used.

ABS=Absorbance

MDA=Malondialdehyde

1- ((ABS 532+TBA)-(ABS 600+TBA)-(ABS 532-TBA)-(ABS 600-TBA)= A

2- ((ABS 440+TBA)-(ABS 600+TBA)x0,0971=B

3- nmolMDA/ml= (A-B/157000)x106

RNA Isolation and qRT-PCR Analysis

All of the plant samples taken after the stress treatments were kept in refrigerator at -80˚C until the RNA isolation procedure. For each of stress conditions, 3 biological replications were employed. For RNA isolation from the plant samples, the protocol of manufacturer company was followed (Total RNA Extraction Kit; Vivantis Malaysia). RNA concentration and amount were determined using the Spectrophotometer (Thermo Multiskan™ GO Microplate) with nano-drop feature. Moreover, RNA samples were swiped into 1% agarose gel. cDNA synthesis was determined using M-MuLV Reverse Transcriptase RNase H- (Vivantis, Malaysia) in accordance with the guidelines provided by the manufacturer. For real-time implementations, Thermo SYBR Green Master Mix and Real-Time PCR (LightCycler® Nano Roche; Mannheim, Germany) were used, and the reaction was performed in following order; at 95°C for 10 min. and then at 95°C for 15 s, 55°C (binding temperature varies depending on the primer) for 30 s and 72°C for 30 s for 40 cycles. Melting curve was obtained by heating the amplicon from 55°C to 95°C. Transcript abundance of PIP2.1 and PIP2.2 was analyzed using specific primers (Baiges et al. 2001). In order to determine the change in expression level, *VvActin* reference gene was used.

Statistical Analysis

The significance of differences was analyzed sing "Independent t-Test" by using JMP 13 software.

RESULTS AND DISCUSSIONS

Relative Moisture Content (RMC)

The effects on relative moisture content are seen in Figure 1 for 'Horozkarası' variety. Accordingly, it was determined that the treatments have statistically significant effect. When compared to control group, the relative moisture content of plants grown in drought treatment was found to significantly decrease (p:<0.05).

In Figure 1, the change in RMC by drought is presented.

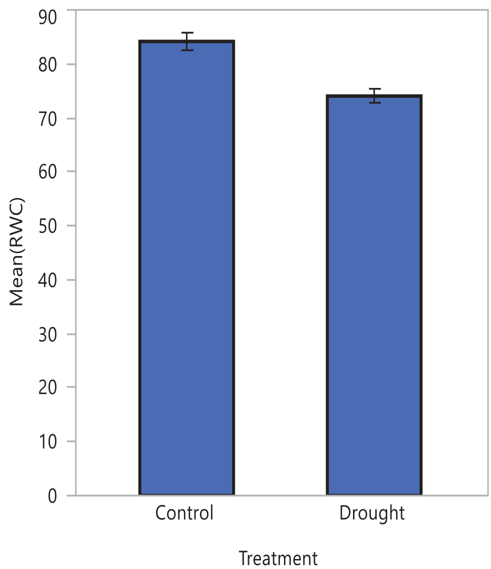


Figure 1. The effects on relative moisture content of 'Horozkarası' variety

Membrane Permeability (MP)

In Figure 2, the effects on membrane permeability for 'Horozkarası' grape variety can be observed.

Accordingly, the treatments were found to have significant effect.

When compared to control, 1% change in membrane permeability of plant grown in drought treatment was found to be statistically significant (p:<0.05).

In figure 2, the change in membrane permeability (%) by drought is presented.

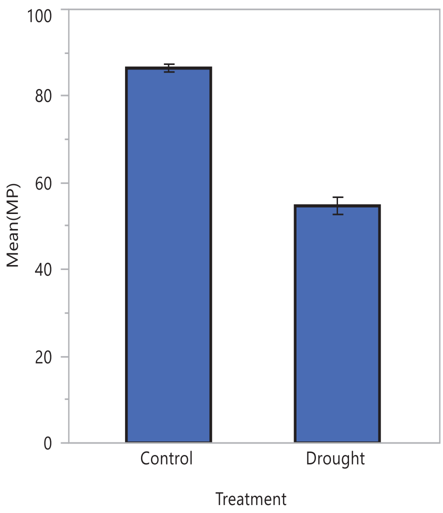


Figure 2. The effects on membrane permeability for 'Horozkarası' grape variety

Lipid Peroxidation (MDA) Level

In Figure 3, the effects on lipid peroxidation level for 'Horozkarası' grape variety can be seen. Accordingly, the treatments were found to have significant effect. It was observed that the increase in MDA level of plant grown in drought treatment was found to be statistically significant ($p:<0.05$). The change in MDA level (nmol ml^{-1}) by drought is presented.

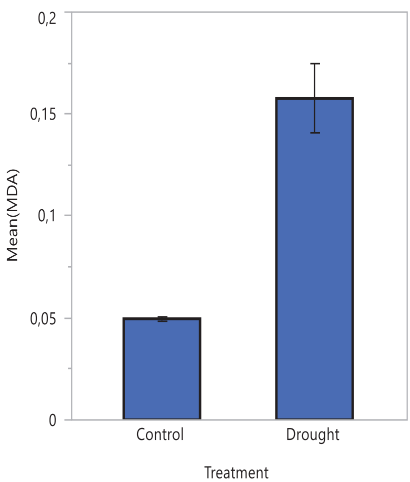


Figure 3. The effects on lipid peroxidation level for 'Horozkarası' grape variety

Aquaporin gene expression

PIP2-1 and PIP2-2 expression levels were examined in root and leaf samples taken from 'Horozkarası' grape variety exposed to stress. While significant decreases were observed in PIP2-1 and PIP2-2 in stress treatment in leaves, increases were observed in expressions of PIP2-1 and PIP2-2 genes in drought treatment in root ($p:<0.05$) (Figure 4). The role of AQPs in regulating plant's water status under water stress is a complex issue, because the expression of different AQP genes may be stimulated, decreased, or unchanged under abiotic stress (Yamaguchi et al., 1992; Maurel 1997; Kirch et al., 2000; Kawasaki et al., 2001). In grapevine, highly vigorous rootstocks have higher fine-root hydraulic conductivity partly due to the higher aquaporin expression and activity (Gambetta et al., 2012). AQPs in plants often show a tissue/organ-specific expression (Tyerman, Niemietz & Bramley, 2002). Chemical signaling such as ABA and hydraulic signaling via aquaporins regulate the stomatal conductance. Relative abundance of transcripts in roots and leaves strongly depended on which AQP and the treatment given (Galmés et al., 2007). In non-stressed plants, PIP1.1 was more abundantly expressed in the roots compared to leaves, consistent with the observations described for other PIPs in the roots of other varieties (Weig et al., 1997; Jang et al., 2004). Plants exposed to short-term water stress showed an enhanced ratio of root-to-leaf AQP expression, particularly for the moderate stress treatment. In study of Aroca et al. (2012), it was emphasized that plasma membrane intrinsic protein (PIP) sub-group played important role in absorbing water from the soil. It was found that own-rooted grapevine cultivars that differ in their response to soil water deficits via differences in the regulation of the leaf water potential also vary in their root response to water soil deficits in terms of aquaporin expression (Vandeleur et al., 2009). In parallel with the results obtained in present study, it was observed that the expression of aquaporin genes in the leaves decreased to limit water loss via transpiration, whereas the expression of the same aquaporin genes increased in the roots to enhance water uptake to avoid plant water constraints when water deficits occurred.

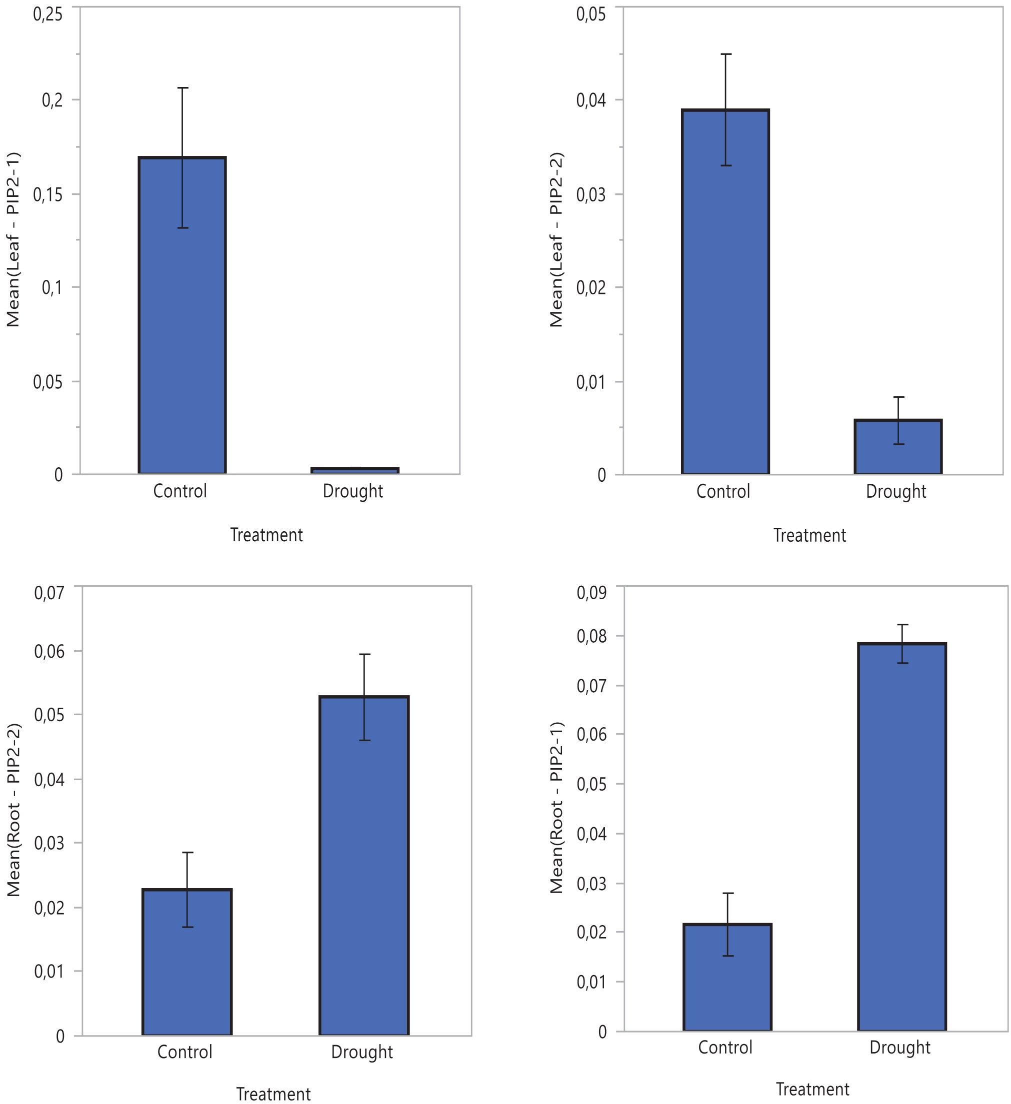


Figure 4. PIP2-1 and PIP2-2 expression levels in root and leaf samples taken from 'Horozkarası' grape variety

CONCLUSIONS

In this study, it was determined that the 'Horozkarası' grape variety, which has successfully adapted to low annual precipitation levels and arid summer conditions of Southeastern region of Turkey, gave physiological, biochemical and gene-level responses to drought and well-adapted to semi-arid regions. For this reason, it is believed to have potential to contribute to the breeding studies on developing varieties that have high tolerance to the drought.

REFERENCES

Aganchich B., Wahbi S., Loreto F., Centrito M., 2009. Partial root zine drying: regulation of phoyosynthetic limitations and antioxidant enzymatic activities in young olive (*Olea europaea*) saplings. Tree Physiol. 29, 685–696.

Alexandros Beis, Angelos Patakas, 2012. Relative contribution of photoprotection and anti oxidative mechanisms to differential droguth adaptaion ability in grapevines; Environmental and Experimenatal Botany 78, 173 – 183.

Chaumont M, Morot-Gaudry J-F, Foyer CH., 1994. Seasonal and diurnal changes in photosynthesis and carbon partitioning in *Vitis vinifera* leaves in vines with and without fruit. Journal of Experimental Botany 45, 1235–1243. doi:10.1093/jxb/45.9.1235.

Flexas J, Briantais J-M, Cerovic Z, Medrano H, Moya I., 2000. Steady-state and maximum chlorophyll fluorescence responses to water stress in grapevine leaves: a new remote sensing system. Remote Sensing of Environment 73, 283–297. doi:10.1016/S0034-4257(00)00104-8

Fukaki H, Tasaka M., 2009. Hormone interactions during lateral root formation. J. Plant Mol. Biol. 69: 437-449

Galmés J., Pou A., Alsina M. M., Tomàs M., Medrano H., Flexas J., 2007. Aquaporin expression in response to different water stress intensities and recovery in Richter-110 (*Vitis* sp.): relationship with ecophysiological status. Planta, 226(3); 671–681.

Gürsöz S., 1993. GAP Alanına Giren Güneydoğu Anadolu Bölgesi Bağcılığı Ve Özellikle Şanlıurfa İlinde Yetiştirilen Üzüm Çeşitlerinin Ampelografik Nitelikleri İle Verim Ve Kalite Unsurlarının Belirlenmesi Üzerinde Bir Araştırma. Türkiye 2. Ulusal Bahçe Bitkileri Kongresi, Cilt 2., ADANA.

Hayes M.A., Davies C., Dry I.B., 2007. Isolation, functional characterization andexpression analysis of grapevine (*Vitis vinifera* L.) hexose transporters:differential roles in sink and source tissues. J. Exp. Bot. 58, 1985–1997

Jang JK, Kim DG, Kim YO, Kim JS, Kang H., 2004. An expression analysis of a gene family encoding plasma membrane aquaporins in response to abiotic stresses in Arabidopsis thaliana. Plant Mol Biol 54:713–725.

Jiang. M., Zhang J., 2002. Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. J. Exp. Bot. 53, 2401–2410.

Lima A.L.S., DaMatta F.M., Pinheiro H.A., Totola M.R., Loureiro M.E., 2002. Photochemical responses and oxidative stress in two clones of Coffea canephora under water deficit conditions. Environ. Exp. Bot. 47, 239–247.

Lovisolo C, Schubert A., 2006. Mercury hinders recovery of shoot hydraulic conductivity during grapevine rehydration: evidence from a whole-plant approach. New Phytol 172:469-478.

Lovisolo C, Tramontini S, Flexas J, Schubert A., 2008. Mercurial inhibition of root hydraulic conductance in *Vitis* spp. rootstocks under water stress. Environ Exp Bot 63: 178–182.

Maroco JP, Rodríguez ML, Lopes C, Chaves MM., 2002. Limitations to leaf photosynthesis in field-grown grapevine under drought – metabolic and modelling approaches. Functional Plant Biology 29, 451–459. doi:10.1071/PP01040

Ozkur O., Ozdemir F., Bor M., Turkan I., 2009. Physiochemical and antioxidant responses of the perennial xerophyte Capparis ovata Desf. to drought. Environ. Exp. Bot. 66, 487–492.

Peleg Z, Blumwald E., 2011. Hormone balance and abiotic stress tolerance in crop plants. J. Current Opinion Plant Biol. 14:290-295.

Pongrácz DP., 1983. Rootstocks for grapevines. David Philip Publisher, Cape Town, South Africa.

Pou A., Medrano H., Flexas J., Tyerman S.D., 2013. A putative role for TIP and PIP aquaporins in dynamics of leaf hydraulic and stomatal conductance ingrapevine under water stress and re-watering. Plant Cell Environ. 36, 828–843.

Ramachandra R.A., Chaitanya K.V., Jutur P.P., Sumithra K., 2004. Differential antioxidative responses to water stress among five mulberry (*Morus alba* L.) cultivars. Environ. Exp. Bot. 52, 33–42.

Sanchez-Diaz M., Tapia C., Antolin M.C., 2007. Drought-induced oxidative stress in different Canarian laurel forest tree species growing under controlled conditions. Tree Physiol. 27, 1415–1422.

Schachtman DP, Goodger JQ., 2008. Chemical root to shoot signalling under drought. Trends Plant Sci. 13: 281-287.
Schachtman DP, Goodger JQ., 2008. Chemical root to shoot signalling under drought. Trends Plant Sci. 13: 281-287.
Sofo A., Dichio B., Xiloyannis C., Masia A., 2005. Antioxidant defences in olive trees during drought stress: changes in activity of some antioxidant enzymes. Funct. Plant Biol. 32, 45–53.

Surbanovski N., Grant O.M., 2014. The emerging role of aquaporins in plants'tolerance of abiotic stress. In: Paraviz Ahmad, P., Rasool, S. (Eds.), Emerging Technologies and Management of Crop Stress Tolerance, Volume 2- A Sustainable Approach. Elsevier Inc., 431–447.

Vandeleur R.K., Mayo G., Shelden M.C., Gilliham M., Kaiser B.N., Tyerman S.D., 2009. The role of plasma membrane intrinsic protein aquaporins in watertransport through roots: diurnal and drought stress responses reveal differentstrategies between isohydric and anisohydric cultivars in grapevine. PlantPhysiol. 149, 445–460.

Wang W.B., Kim Y.H., Lee H.S., Kim K.Y., Deng X.P., Kwak S.S., 2009. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant Physiol. Biochem. 47, 570–577

Weig A, Deswarte C, Chrispeels MJ., 1997. The major intrinsic protein fanily of Arabidopsis has 23 members that form three distinct groups with functional aquaporins in each group. Plant Physiol 114:550–555.

Wilkinson S., 1999). pH as a stress signal. Plant Growth Regulation. 29:87-99.

Zarrouk O., Garcia-Tejero I., Pinto C., Genebra T., Sabir F., Prista C., Chave M.M., 2015. Aquaporins isoforms in cv. Touriga Nacional grapevine under water stress and recovery—Regulation of expression in leaves and roots. Agricultural Water Management. http://doi.org/10.1016/j.agwat.2015.08.013.

Zhang J., Kirkham M.B., 1996. Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytol. 132,361–373.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

OCCURRENCE OF *ISARIOPSIS* LEAF SPOT OR BLIGHT OF *VITIS RUPESTRIS* CAUSED BY *PSEUDOCERCOSPORA VITIS* IN TURKEY

Fatih Mehmet TOK, Sibel DERVIŞ

Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, 31040 Antakya, Hatay, Turkey

Corresponding author email: ftok@mku.edu.tr

Abstract

In mid-autumn 2013, a disease of Vitis rupestris affecting 30 to 50% of the plants was observed in an orchard of the Kırıkhan district of Hatay province in the east Mediterranean region of Turkey. Symptoms included presence of initially tiny and angular chlorotic halos but latterly large black spots. On the spots, cercosporoid structures were present. Based on cultural and morphological characteristics of the consistently isolated fungus and pathogenicity tests, the causal agent of this leaf spot or blight was identified as Pseudocercospora vitis. This appears to be the first report of P. vitis on V. rupestris in Turkey. Since Isariopsis leaf spot caused by P. vitis can also affect grapevines (V. vinifera), it might negatively impact orchards and vineyards in the future and might be considered a potential threat to Turkish grapevine production.

Key words*: leaf blight, Vitis rupestris, Pseudocercospora vitis, Mycosphaerella personata.*

INTRODUCTION

Grapevine is a widely planted and economically important crop in Turkey, hosting valuable grape germplasm resources with a total area under production of 467,093 hectares, producing 4,175,356 tonnes of grapes (FAO, 2014). In Kırıkhan and Hassa districts of Hatay, a coastal southeastern province located in the east Mediterranean region having about 20% of the grapevine growing areas of the country, some family farmers cultivate *Vitis rupestris*, a grape species commonly known as an American grapevine rootstock.

In mid-autumn 2013, a disease of *V. rupestris* plants was observed in an orchard of the Kırıkhan district of Hatay province in the east Mediterranean region of Turkey.

This study represents the first attempt to identify and characterize a cercosporoid species causing *Pseudocercospora* leaf spot, a disease of the aerial part of the *V. rupestris* vines, in Turkey using morphological and pathogenicity approaches.

MATERIALS AND METHODS

In October 2013, symptoms consisting of necrotic leaf spots were observed on *V. rupestris* cv. du Lot grown in an orchard in the Kırıkhan District and affected leaves sampled. Leaf tissues bordering these lesions surface disinfested with NaOCl (1%) for 2 min, rinsed in sterile water, blotted dry, and plated onto potato dextrose agar (PDA).

Isolated fungus was transferred to V-8 juice agar and malt extract agar (MEA). Plates were incubated for 30 days at 25°C under NUV light and a 12-h light/dark photoperiod for morphological examination. Morphological and colony characteristics of the fungus were examined.

Pathogenicity tests were performed on 10 1-year-old potted plants of grapevine (*V. rupestris*) cv. du Lot. Inoculations were performed by spraying a conidial suspension (3.0×10^4 conidia/ml) prepared in sterile water by harvesting conidia from 2-week-old cultures on V8 agar until runoff onto the leaves of healthy seedlings using manual pressure sprayer. Three control plants were sprayed with sterile water. Inoculated and control plants were covered with plastic bags to maintain a relative humidity of 100% for 48 h and then transferred to a greenhouse.

RESULTS AND DISCUSSIONS

Leaf spots were initially tiny, irregular to angular chlorotic halos or patches on both leaf surfaces - abaxial (Figure 1) and adaxial (Figure 2), and as the disease progressed, they were progressively turned purplish brown to black lesions. These spots later coalesced (amphigenous or confluent) reaching to 2 cm in diameter mostly with a serpentine and well defined outline encircled with dark borders or chlorotic halos on the upper leaf surfaces and became brittle with age (Figure 3). On the corresponding lower leaf surfaces, only the narrow centers of these lesions were brown to black but surrounding tissue was a large necrotic area (Figure 4). These coalesced spots caused leaf blight and premature defoliation. The disease incidence approached 30 to 50% on vines (cv. du Lot). The leaf spots were more severe on the leaves near the ground and progressed to the upper leaves. When infected tissue was examined under a stereomicroscope, typical cercosporoid hyphomycete structures were observed within the lesions on both leaf sides but mostly on the upper side.

A slow-growing fungus was consistently isolated from the affected tissues after 5 days of incubation at 25°C.

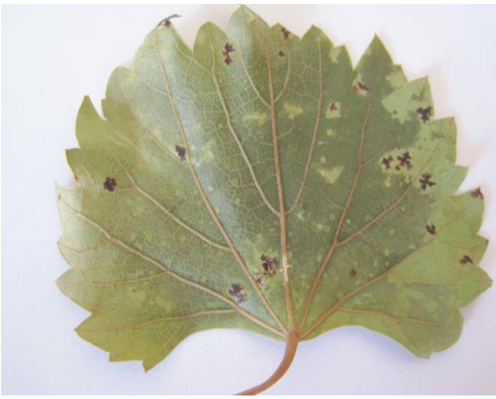


Figure 1. Irregular to angular chlorotic halos on the abaxial leaf surface of *Vitis rupestris* du Lot

Colonies were gray with black stromatic structures in the centers on the upper side (Figure 5) and dark green on the underside. The fruiting structures were slender, black, bristlelike synnemata (200-500 µm long) bearing pale olivaceous to pale brown, elongate conidia (25 to 100 × 4 to 7 µm) 3 to 7 transverse septa and no longisepta (Figure 6). Morphological characteristics of the fungus were consistent with previous descriptions of *Pseudocercospora vitis* (Lév.) Speg. (Ascomycetes, Mycosphaerellales), the anamorph of *Mycosphaerella personata* Higgins as described by Ellis (1971) and Harvey and Wenham (1972).

Figure 2. Initial symptoms of Isariopsis leaf spot on the adaxial leaf surface of *Vitis rupestris* du Lot

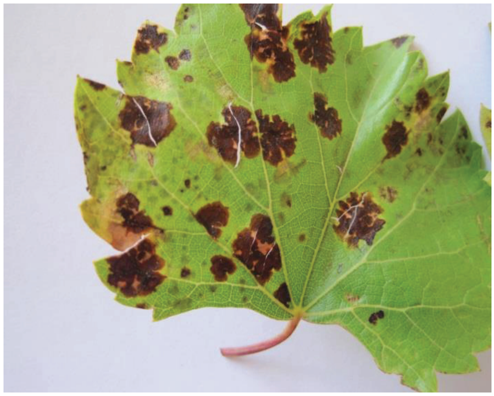


Figure 3. Leaves of *Vitis rupestris* du Lot, with leaf spots on abaxial surface, late in the season

Pathogenicity was confirmed by fulfilling Koch's postulates. Necrotic spots appeared on the inoculated leaves 20 days after inoculation, and were identical to the ones observed in the field. Disease incidence on inoculated leaves varied from 30% to 91% and severity from 2 to 3 to 3 to 6 lesions per leaf. No symptoms were observed on control plants. Fungal colonies morphologically identified as *P. vitis* were reisolated from lesions on inoculated leaf tissues, fulfilling Koch's postulates. Control plants remained symptomless.

Figure 4. Leaves of *Vitis rupestris* du Lot, with leaf spots on adaxial surface, late in the season

Based on these results, the disease was identified as *Pseudocercospora* or *Isariopsis* leaf spot of *V. rupestris* caused by *P. vitis*. Since the pathogen was named *Isariopsis clavispora* formerly, the disease is still often referred to as *Isariopsis* leaf spot. Its occurrence primarily reported on *V. vinifea* and vines of wild species in the United States and throughout the warmer grape-growing areas of the world under one or another of its several synonyms (Pearson, 1998).

On *V. rupestris*, it was only recorded in Italy (Chupp, 1953) and Kansas (Anonymous, 1960) based on Farr and Rossman (2017).

To our knowledge, this is the first report of *P. vitis* on this host plant species in Turkey. Therefore, this present report is considered one of the few reports of this grapevine disease on *V. rupestris* in the world.

The appearance of the disease was more frequent at the end of the plant's vegetative cycle, in this American cultivar.

The main damage resulting from the attack of the pathogen was the premature fall of leaves, which caused a weakening of the plant and reduction of production in the following year, in 2014.

Cultural practices that increase air circulation such as shoot positioning and thinning may aid in management of the disease.

Figure 5. Colony of *Pseudocercospora vitis* after 30 days growth on potato dextrose agar

Figure 6. Conidia of *Pseudocercospora vitis*

CONCLUSIONS

A prominent leaf spot disease was found to occur on *V. rupestris* in Kırıkhan district of Hatay province in Turkey. A leaf spotting hyphomycete, *P. vitis,* was isolated, identified and proved to be pathogenic on grapevine. Associated symptoms were described and illustrated. Keeping vines healthy, destroying crop residues and spraying with standard fungicides (mid to late season) are recommended to perform in the case of severe attacks of the disease.

ACKNOWLEDGEMENTS

We thank Prof. Dr. Gülşen Sertkaya from the Department of Plant Protection at the University of Mustafa Kemal, for her participation in the expeditions and Assoc. Prof. Dr. Önder Kamiloğlu from the Department of Horticultural Science at the University of Mustafa Kemal, for the identification of the plant species.

REFERENCES

Anonymous, 1960. Index of Plant Diseases in the United States. U.S.D.A. Agric. Handb. 165, 1-531.

Chupp C., 1953. Monograph of the fungus genus *Cercospora*. Published by the Author, Ithaca, New York, 667 pages.

Ellis M.B., 1971. Dematiaceous Hyphomycetes. Kew, UK: Commonwealth Mycological Institute.

FAO, 2014. FAOSTAT. Food and Agriculture Organization of the United Nations, Rome, Italy Web. http:// www.fao.org/faostat/en/#data/QC

Farr D.F., Rossman A.Y., 2017. Fungal Databases. Syst. Mycol. Microbiol. Lab., Online publication, ARS, USDA, Retrieved 16 February, 2017.

Harvey I.C., Wenham H.T., 197.) A Fungal Leaf Spot Disease of Grapes *Cercospora vitis* (Lév) Sacco , New Zealand Journal of Botany, 10 (1), 87-96.

Pearson R., 1998. Compendium of Grape Diseases. (A.C. Goheen ed.) St. Paul, Minnesota: American Phytopathological Society.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE USE OF A GC-ELECTRONIC NOSE FOR THE SELECTION OF A WINEMAKING PROTOCOL LEADING TO AN ENHANCED VOLATILE PROFILE IN WINES FROM AROMATIC GRAPE VARIETIES

Arina Oana ANTOCE, George Adrian COJOCARU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Horticulture, Department of Bioengineering of Horti-Viticultural Systems,
59 Mărăşti Blvd., District 1, 011464 Bucharest, Romania

Corresponding author emails: arina.antoce@horticultura-bucuresti.ro; aantoce@yahoo.com

Abstract

The multiple oenological materials used in winemaking protocols have various influences on the final aromatic profile of wine. Therefore, their overall effect on a certain variety is difficult to determine, even when some of the materials are tested separately. To simplify the decision, if the winemaker would simply like to enhance the number or the concentration of the compounds forming the volatile profile of a wine, we propose in this paper a method which uses an electronic nose based on flash gas chromatographic technique to test the effect of a certain winemaking protocol. The test was made on two aromatic Romanian autochthonous varieties, Busuioaca de Bohotin and Tamâioasa românească, each vinified with 3 different winemaking protocols. The volatile profiles for each winemaking protocol and variety were recorded and compared by using multivariate statistical analysis, in order to pair the variety and protocol which can generate a more intense volatile profile.

***Key words**: electronic nose, volatile profile, aromatic wine, Busuioaca de Bohotin, Tamâioasa românească.*

INTRODUCTION

The aromatic profile of a wine is a trait on which many consumers base their choice. For this reason many researchers have taken into account the possibilities to modify the volatile profile of a wine by using various winemaking protocols.

The main influences on the aromatic profile of the wines are imposed by the grape variety (Rocha et al., 2010). However, even for the same grape variety, the styles of wines possible to obtain vary, in accordance with the substances extracted from the skins by maceration (González-Pombo et al., 2014; Lao et al., 1997) and also by the volatile compounds released by enzymes or yeasts (Palmeri and Spagna, 2007; Cabaroglu et al., 2003) from heavier molecules, called aroma precursors, which are usually glycosides of these aroma compounds. Both advanced extraction and aroma release from precursors are achieved by treatments with specific enzymes (Piñeiro et al., 2006) or presence of specific yeasts (Loscos et al., 2007; Hernandez-Orte et al., 2008).

Other influences on aroma profile of a wine are induced by fermentation, when the secondary aroma of the wine is formed (Sumby et al., 2010). Here too we have several influences. The fermentation aroma is mainly determined by the yeast used for the winemaking process (Ubeda Iranzo et al., 2000; Swiegers and Pretorius, 2005; Swiegers et al., 2009; Samoticha et al., 2017), but raw material itself (Ghaste et al., 2015) and the fermentation activator (Marks et al., 2003; Barbosa et al., 2009; Ugliano et al., 2009) may also induce perceivable differences.

When producing wines, even from the same variety, numerous combinations of enzymes, activators and yeast are possible, all being included in what we call a winemaking protocol, and all having various influences in accordance to the grape variety and performed treatments (Piñeiro et al., 2006).

When applying a winemaking protocol, it is difficult to predict how the several oenological material and treatments entailed in this protocol are going to influence the aroma profile. Even if each oenological material is tested separately, it is not certain that the winemaker is able to predict the final result when combining the materials into a certain protocol.

Thus, to simplify the decision, if the winemaker would simply like to enhance the number or concentration of the components of the volatile profile of a wine, it is possible to test the effect of a certain winemaking protocol by the use of an electronic nose based on flash gas chromatographic technique. In this way, the testing of separate oenological materials which are part of a winemaking protocol is not anymore necessary, this method of evaluating only the final profile saving time and effort.

MATERIALS AND METHODS

Wines of aromatic grape varieties Tamâioasa românească and Busuioaca de Bohotin were produced at industrial scale in volumes of 300 hl. Each variety was vinified by using 3 different protocols (technological schemes), making use of specific enzyme treatments and specific yeast and fermentation activators. As the winemaking protocols are based on commercial products, in order to avoid conflict of interests, the brand names and producers are not disclosed in this paper.

The wine samples prepared are generically described in Table 1.

The volatile profile of each wine variant was determined by a flash gas chromatograph with two short different polarity columns, working on the principle of the electronic nose.

The apparatus from the Alpha-MOS, France, is fitted with a DB-5 (non-polar) and a BD-1701 (slightly polar) 2 m columns and for the chromatographic peak recording two flame ionization detectors, one for each column, thus resulting two simultaneous chromatograms with an acquisition time of 40 s.

Table 1. Protocol description and codification of experimental wines

Wine sample	Experimental protocol					
	Variety	Protocol code	Extraction enzyme	Clarifying enzyme	Fermentation activator	Yeast
BB_AP	Busuioaca de Bohotin	AP	E1	C1	A1	Y1
BB_ED		ED	E2	no	A2	Y2
BB_LA		LA	E3	C3	A3	Y3
TR_AP	Tamâioasa româneasca	AP	E1	C1	A1	Y1
TR_ED		ED	E2	no	A2	Y2
TR_LA		LA	E3	C3	A3	Y3

Each wine sample was injected in the e-nose in triplicate, using the method developed in our laboratory for wines (Antoce and Namolosanu, 2011; Antoce et al., 2015). The main parameters are: injection volume 2500 µl, trap (40°C, pre-purging time 5 s, preheating 20 s, baking 60 s, desorption temperature 250°C), column (heating from 40°C to 200°C with 5°C/s increment, maintaining 2 s the initial and 5 s the final temperature), injection at 200°C, detector temperature 220°C.

The data recording and processing is based on the AlphaSoft version 12.42 and Arochembase library.

Several multivariate statistical analyses are used and compared for data processing, such as the Principal Component Analysis (PCA), which can show the differences in the volatile profiles in accordance to the winemaking protocol used (evaluate discrimination performance), the Discriminant Factorial Analysis (DFA), which can be used to separate in clusters the samples with similar volatile profiles and when necessary classify the unknown samples into these clusters, and finally to determine odor distances from one cluster used as reference by Statistical Quality Control (SQC) analysis.

RESULTS AND DISCUSSION

The electronic nose is able to discriminate the samples in accordance to their raw material – the grape variety – but also to the winemaking protocol used for their preparation, being capable to identify the samples with enhanced volatile profile.

a) Discrimination of grape variety and winemaking protocol by PCA

By applying a Principal Component Analysis a good discrimination (with a discrimination index of 83) can be obtained of the wine sample clusters containing the same variety and winemaking protocol (Figure 1).

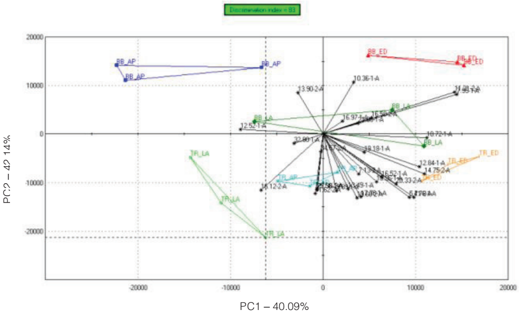


Figure 1. PCA diagram showing the discrimination by the electronic nose of the groups of samples by grape varieties (BB-dark colours and TR-light colours) and winemaking protocols (AP- light/dark blue, ED- light/dark red, LA- light/dark green)

It can be seen that the PC2 axis, which accounts for 42% of the data variability, includes the variables which are dependent on the variety; thus, the clusters of Busuioaca are placed in the upper part of the diagram, while the clusters of Tamaioasa are placed in the lower part of the diagram.
The winemaking protocols are mostly discriminated by the variables included in the PC1, which accounts for 48% of the data variability. Irrespective of the variety, the samples obtained with winemaking protocols AP and LA are placed on the left and those with winemaking protocol ED are placed on the right of the diagram.
This behaviour is most likely determined by the common volatile substances produced by the specific yeast used in each protocol, and not by the specific enzymes used, which also had some influences on the compounds extracted form the grapes or released from grape aromatic precursors.

The third PC only accounts for 4.96% of the variability (Figure 2) and is probably related to the differences induced both by the grape variety and the wine protocol - thus most likely by the type of the enzymes used in each winemaking process.

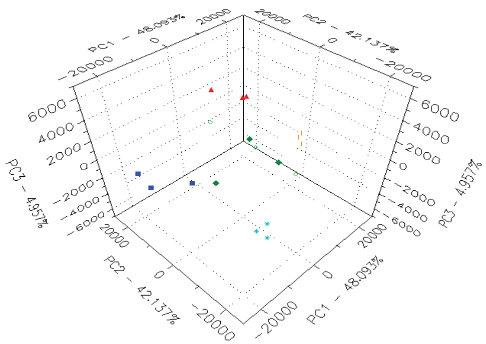


Figure 2. 3D-PCA diagram showing the discrimination by the electronic nose of the groups of samples by grape varieties and winemaking protocols

From Figure 1 we can also see that the most discriminant chromatographic peaks for Busuioaca grape variety are 13.90-2A and 10.36-1A, which were identified as ethyl 2-methyl-butanoate and buthyl acetate, respectively, while for Tamaioasa there were more peaks, among which we can cite 25.58-2A (linalool), 11.62-2A (ethyl butanoate), 13.49-1A (isoamyl acetate), 17.76 1A (β-pinene), 19.60-2A (ethyl hexanoate).
Even more peaks contributed to the discrimination of the winemaking protocol, their importance in discrimination being difficult de determine.

b) Discrimination of grape variety and winemaking protocol by DFA

A similar behaviour of the sample clusters as that described in the PCA diagrams can be observed when the Discriminant Factor Analysis is applied (Figure 3). Most of the variation is included in the DF 1 (79.53%), which is related to the grape variety.
The variation determined by the winemaking protocol (the yeast and to a certain degree the enzymes) is included mainly in the DF2 (16.75%) and DF3 (2.74) factors.

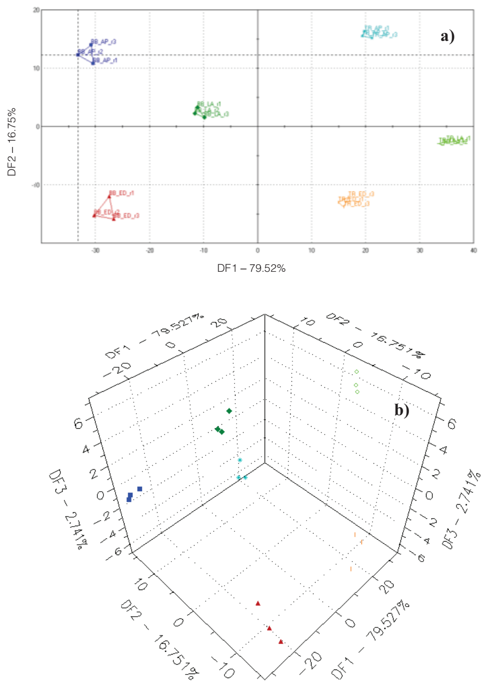


Figure 3. FDA (3a) and 3D-FDA (3b) diagram showing the discrimination by the electronic nose of the groups of samples by grape varieties and winemaking protocols

c) Discrimination of the influences induced by the winemaking protocols for Busuioaca de Bohotin

When only samples obtained based on the same variety are compared, the variability is reduced and the results show only the influence of the winemaking protocol.
The DFA analysis for the Busuioaca de Bohotin allows for discrimination among the samples clusters, but this time clusters are placed much closer to one another (Figure 4).

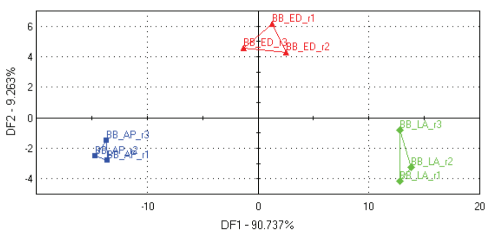


Figure 4. Discrimination by DF Analysis of the groups of Busuioaca de Bohotin samples obtained by different winemaking protocols

The influence of the winemaking protocol for Busuioaca de Bohotin is not much, most of the variability being included in the DF1=90.74%. The variability included in DF2 (9.26%) is mostly related the winemaking protocol ED, which is differentiated by the variables included in this function.
The ED protocol stands out also when it comes to the odor intensity, which was the highest among the winemaking protocols (Figure 5).

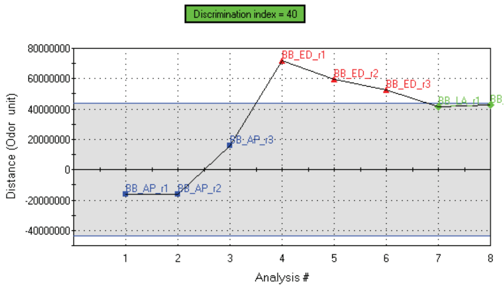


Figure 5. Odor intensity distances of Busuioaca de Bohotin samples obtained by different winemaking protocols

The clusters of BB samples produced with the ED protocol clearly differentiated from the samples produced with protocols AP and LA, when the odor intensity assessed by measuring the odor distances among the samples were determined.
This higher concentration of volatile substances in the wine profile could be a good indication of fermentation with a yeast more effective in producing secondary metabolites, but this does not necessarily indicate a better aromatic profile. On the contrary, the wines produced with the ED protocol ranked last among the three protocols on a sensory evaluation based on the OIV score sheet: BB-ED = 84, BB-LA = 88 and BB-AP = 90 points.

d) Discrimination of the influences induced by the winemaking protocols for Tamâioasa romaneasca

In the case of Tamaioasa romaneasca the winemaking protocol had a higher influence than in the case of Busuioaca de Bohotin (Figure 6). The variability induced by the winemaking protocol was 83.22 included on DF1 and 16.78% in DF2, the last one being mostly related to the LA protocol.

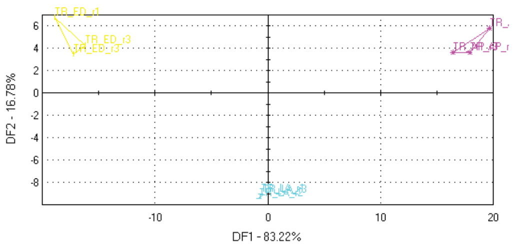


Figure 6. Discrimination by DF Analysis of the groups of Tamaioasa romaneasca samples obtained by different winemaking protocols

For this variety, the highest odor intensities were also displayed by the wines based on ED protocol (Figure 7), confirming that this protocol is clearly different and identifiable by the E-nose, irrespective of the raw material used.

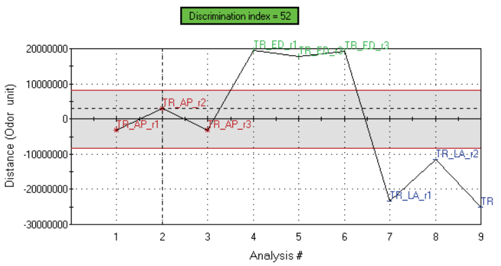


Figure 7. Odor intensity distances among the groups of Tamaioasa romaneasca samples obtained by different winemaking protocols

The sensory evaluation based on the OIV score sheet showed the following ranking: TR-ED and TR-LA = 86 points and TR-AP = 92 points. This shows that the high intensity volatile profile was not preferred by the winetasters, but neither was the lowest intensity profile (LA protocol).

CONCLUSIONS

Based on the evaluation of the volatile profiles of the wines produced from the two aromatic varieties using three different winemaking protocols, the following conclusions can be drawn:

- The variety is most likely discriminated by the electronic nose based on the terpenic volatile profile of each variety, but also by other esters and acetates either from the primary or secondary aroma.
- The winemaking protocol has two main components, with different influences: the enzyme influences the primary aroma by changing the level of extraction of several aromatic compounds and also the degree of release of the aromatic compounds from precursors, while the yeast influences the secondary/fermentation aroma, by the esters and acetates that it forms during fermentation. The yeast has the main influence in the aromatic profile of wines obtained from the same variety.
- Irrespective of the grape variety used, the ED winemaking protocol stands out from the three different protocols, with larger distances measured in odor intensity units, compared to the other two protocols. A higher concentration of the volatile substances measured for this winemaking protocol may indicate a higher aroma intensity which can be perceived sensorially, but it may not necessary mean that the consumers will prefer the resulted wines. It is however a good indication that the wines will be more intense in the nose, a trait that some consumers like.
- For the final protocol selection the results of e-nose testing should always be correlated with the sensory analysis results.
- This type of e-nose analysis may be particularly useful when more than three winemaking protocols are under evaluation and preparation of many wines on industrial scale may be not an economical option.

REFERENCES

Antoce O.A., Nămoloşanu I.C., 2011, Rapid and precise discrimination of wines by means of an electronic nose based on gas-chromatography, Revista de Chimie, Vol. 62, No. 6, 593-595.

Antoce O.A., Stroe M.V., Cojocaru G.A., 2015, Tentative application of an electronic nose to the study of the parentage of Romanian grape varieties Sarba and Alb Aromat, Agriculture and Agricultural Science Procedia, Elsevier, 6 (2015), 110 – 117.

Barbosa C., Falco V., Mendes-Faia A., Mendes-Ferreira A., 2009, Nitrogen addition influences formation of aroma compounds, volatile acidity and ethanol in nitrogen deficient media fermented by Saccharomyces cerevisiae wine strains. Journal of Bioscience and Bioengineering, 108(2), pp. 99–104.

Cabaroglu, T., Selli, S., Canbas, A., Lepoutre, J. P., & Günata, Z., 2003, Wine flavor enhancement through the use of exogenous fungal glycosidases. Enzyme and Microbial Technology, 33, 581–587.

Ghaste M., Narduzzi L., Carlin S., Vrhovsek U., Shulaev V., Mattivi F., 2015, Chemical composition of volatile aroma metabolites and their glycosylated precursors that can uniquely differentiate individual grape cultivars, Food Chemistry, Volume 188, 309-319.

González-Pombo P., Fariña L., Carrau F., Batista-Viera F., Brena B. M., 2014, Aroma enhancement in wines using co-immobilized Aspergillus niger glycosidases, Food Chemistry, Volume 143, 185-191.

Hernandez-Orte, P., Cersosimo, M., Loscos, N., Cacho, J., Garcia-Moruno, E., Ferreira, V., 2008, The development of varietal aroma from non-floral grapes by yeasts of different genera, Food Chemistry, 107, 1064–1077.

Lao, C., Lo´pez Tamanes, E., Lamuela Raventos, R. M., Buxaderas, S., Torre Boronat, M., 1997, Pectic enzyme treatment effects on quality of white grape musts and wines. Journal of Food Science, 62, 1142–1144.

Loscos, N., Hernandez-Orte, P., Cacho, J., Ferreira, V., 2007, Release and formation of varietal aroma compounds during alcoholic fermentation from non-floral grape odorless flavor precursors fractions, Journal of Agricultural and Food Chemistry, 55, 6674–6684.

Marks V., K van der Merwe G., van Vuuren H.J., 2003, Transcriptional profiling of wine yeast in fermenting grape juice: regulatory effect of diammonium, FEMS Yeast Research, Volume 3, Issue 3, 269-287.

Palmeri, R., Spagna, G., 2007, Beta-glucosidase in cellular and acellular form for winemaking application, Enzyme and Microbial Technology, 40(3), 382–389.

Piñeiro Z., Natera R., Castro R., Palma M., Puertas B., Barroso C.G., 2006, Characterisation of volatile fraction of monovarietal wines: Influence of winemaking practices, Analytica Chimica Acta, Volume 563, Issues 1–2, 165-172.

Rocha S. M., Paula Coutinho, Elisabete Coelho, António S. Barros, Ivonne Delgadillo, Manuel A. Coimbra, 2010, Relationships between the varietal volatile composition of the musts and white wine aroma quality. A four year feasibility study, LWT - Food Science and Technology, Volume 43, Issue 10,. 1508-1516.

Samoticha J., Wojdyło A., Chmielewska J., Politowicz J., Szumny A., 2017, The effects of enzymatic pre-treatment and type of yeast on chemical properties of white wine, LWT - Food Science and Technology, Volume 79, June 2017, 445-453.

Sumby, K. M., Grbin, P., & Jiranek, V., 2010. Microbial modulation of aromatic esters in wine: Current knowledge and future prospects, Food Chemistry, 121, 1–16.

Swiegers J. H., Kievit R. L., Siebert T., Lattey K. A., Bramley B. R., Francis I. L., King E. S., Pretorius I. S., 2009, The influence of yeast on the aroma of Sauvignon Blanc wine, Food Microbiology, Volume 26, Issue 2, April 2009, 204-211.

Swiegers J. H., Pretorius I. S., 2005, Yeast modulation of wine flavor, Advances in Applied Microbiology, 57(05), 131–75.

Ubeda Iranzo J.F., González Magaña F., González Viña M.A., 2000, Evaluation of the formation of volatiles and sensory characteristics in the industrial production of white wines using different commercial strains of the genus Saccharomyces, Food Control, Volume 11, Issue 2, 143-147.

Ugliano M., Fedrizzi B., Siebert T., Travis B., Magno F., Versini G., Henschke PA., 2009, Effect of nitrogen supplementation and Saccharomyces species on hydrogen sulfide and other volatile sulfur compounds in shiraz fermentation and wine, J. Agric. Food Chem. ,57(11), 4948-55.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCHES ON SITUATION AND TRENDS IN CLIMATE CHANGE IN SOUTH PART OF ROMANIA AND THEIR EFFECTS ON GRAPEVINE

Georgeta Mihaela BUCUR, Liviu DEJEU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: mihaela_g_savu@yahoo.com

Abstract

In this study we analyzed data from three meteorological stations situated in Romania's south part (Bucharest, Constanta and Craiova), in the period 1977-2016. There were calculated several primary climate parameters (annual average temperature; average temperature in the growing season; average temperature in summer; average maximum temperature in the warmest month; average minimum temperature in the coldest month; annual precipitations; precipitations in summer), and bioclimatic indices (Huglin Index, Winkler Index and Cool Night Index). It has also pursued the evolution in time (19 years), of Feteasca regala, the most common grapevine cultivar in Romania, in close connection with the evolution of climatic parameters. In the period studied, it was found a highly significant trend for average temperatures (annual, during the growing season, summer, and maximum) and Huglin and Winkler indices.The results show the role of the variability, from year to year, of the warmer temperature, which affects grape production and its quality. The increase trend of the grapes sugar content was found to be highly significant (r = 0.809^{***}). Reducing titratable acidity of the must under 4 g/L, observed during the last decade, requires acidification measures.

Key words: *grapevine, climate change, effects, trends.*

INTRODUCTION

Vitis vinifera L. is a species which is very sensitive to climate change, considered to be a bioindicator.

Studies in recent decades have highlighted, in most vineyards, significant heating influence on the development of grapevine phenophases, on the main physiological processes, vegetative growth, grape production and quality (Jones et al., 2005; Cotea et al., 2008; Ranca et al., 2008; Irimia et al., 2015).

Climate change, particularly the temperature, influences the composition of grapes and increased sugars accumulation, acidity reduction, the content of anthocyanins in grape skins and the flavor precursors (Cichi et al., 2006; Palliotti et al., 2015).

In the main vineyards of the country, there was found an increase in average air temperature in the period 2000 - 2010, with values between 0.7 and 2.1ºC. The biggest differences were recorded in the vineyards Dealu Mare, Targu Bujor and Murfatlar, highlighting their tendency to aridity (Burzo, 2014).

Following global warming, there was found a change in the evolution of the annual biological cycle of grapevine, with completion faster phenophases (veraison and maturation of the grapes), which is often forced, and with significant consequences on the product quality, which are not always positive (Rotaru et al., 2013).

Grape maturation is accelerated by high temperatures. Sugar accumulation increases with temperature, but certain secondary metabolites such as anthocyanins and aroma precursors are adversely affected by high temperatures.

The annual sequence of phenological stages of grapevine is commonly observed to be accelerated with an increase in temperature (Duchêne and Schneider, 2005).

High concentrations of sugars in berries are not due to photosynthesis and their translocation from leaves and woody parts of the vine, but to water loss through evaporation (Keller, 2015).

Lowering the titratable acidity of the must under 4.0 g/L as a result of global warming requires the addition of tartaric acid to produce balanced wines and to enhance microbiological stability, causing a more expensive winemaking process.

According to the projections of the Intergovernmental Panel on Climate Change (IPCC, 2014), which considers a temperature increase of 1 - 3.7ºC by the end of the century, it is necessary to elaborate strategies for the adaptation and mitigation of climate change.

The objective of the current paper is to present and to evaluate the situation and trends of climate change for the period 1977 – 2016 in three centers situated in Romania's south part (Bucharest, Constanta and Craiova) and their effects on grapevine.

MATERIALS AND METHODS

We used observation data with a complete daily series from 1977-2016 of 3 meteorological stations situated in Romania's south part (Bucharest, Constanta and Craiova).

There were calculated and analysed 10 primary climate parameters and bioclimatic indices (Table 1): annual average temperature; average temperature in the growing season (IV-X); average temperature in summer (VI-VIII); average maximum temperature in the warmest month (July): average minimum temperature in the coldest month (January); annual precipitations; precipitations in summer; Huglin Index, Winkler Index and Cool Night Index.

Table 1. List of climatic parameters and bioclimatic indices

Variable	Description (Equation)	Months
T average I-XII (ºC)	Annual average temperature	I - XII
T average IV-X (ºC)	Growing season temperature	IV - X
T average VI-VIII (ºC)	Summer temperature	VI - VIII
T maximum VII (ºC)	Average maximum temperature in July	VII
T minimum I (ºC)	Average minimum temperature in January	I
P I-XII (mm)	Annual total precipitation	I - XII
P VI-VIII (mm)	Summer total precipitation	VI - VIII
Huglin Index (ºC units)	Σ[(Tavg-10°C) + (Tmax-10°C)] / 2 x k	IV- IX
Winkler Index (ºC units)	Σ[(Tmax+Tmin) / 2 - 10°C]	IV - X
Cool Night Index (ºC)	$Tmin_{sept}$	IX

Vine reaction to climate change was traced during the period 1998-2016 within a 1.0 ha vineyard planted in 1994 at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, at 2.2/1.2 m distance, with Feteasca regala cv (clone 21 Bl) / Kober 5 BB. Vines were trained to a bilateral cordon at 0.7 m and spur pruned with a load of 10 buds / sqm.

The statistical calculations were conducted using Microsoft Excel and interpretation of the data was performed by the methodology presented by Botu and Botu, 2003.

RESULTS AND DISCUSSIONS

Climate change. Analysing primary climate parameters and bioclimatic indices, there were obtained the data presented in Table 2. During the last four decades significant warming trends were observed in the three centers studied. Similar trends were found for Huglin and Winkler indices

According Huglin Index values, the two centers from Muntenia and Oltenia (Bucharest and Craiova) are included in warm temperate climate class (HI + 1), whereas the Black Sea coast (Constanta) is at the limit between the warm temperate and the temperate climate (HI - 1) (Tonietto and Carbonneau, 2004).

The largest increase in temperature trends during 1977 - 2016 were found in Constanta (+ 2.15 ... + 3.65°C).

Following the evolution of annual precipitation reveals a non significant trend for Bucharest and a significant one for Constanta (highy significant trend) and for Craiova (significant trend). Regarding the evolution of the summer precipitations, there was observed a non-significant trend for all three centers.

Cool night Index values (represented by the average minimum temperatures in September) were not significantly affected by climate change, maintaining the areas studied in class climate with very cool nights (CI + 2, Tonietto and Carbonneau, 2004), except for the Black Sea coast (cool nights CI + 1). These conditions ensure, in addition to good grape ripening, also high aromatic and phenolic potential (varieties for red wines).

Following anomalies of average temperatures during the growing season from the multiannual average (Figure 1) there is a significant heating tendency in all 3 studied centers.

Table 2. Trend over the period 1977-2016 and trend per decade
of primary climatic parameters and bioclimatic indices

Location	Variable	Mean and standard deviation	Trend over the period 1977-2016	Trend per decade	R^2
Bucharest (44.75N; 26.11E; 91 m)	T average I-XII (ºC)	11.51±0.75	+ 1.3	+ 0.35	0.223**
	T average IV-X (ºC)	17.97±0.78	+ 1.3	+ 0.35	0.218***
	T average VI-VIII (ºC)	22.37±1.11	+ 2.1	+ 0.52	0.302***
	T maximum VII (ºC)	29.73±1.75	+ 3.1	+ 0.77	0.254***
	T minimum I (ºC)	-5.41±2.46	-	-	NS
	P I-XII (mm)	618.3±149.4	+ 72	+ 18	NS
	P VI-VIII (mm)	193.1±70.8	-	-	NS
	Huglin Index (ºC units)	2337±189	+ 375	+ 93	0.348***
	Winkler Index (ºC units)	1705±168	+ 265	+ 66	0.217***
	Cool Night Index (ºC)	10.52±1.40	-	-	NS
Constanța (44.33N; 28.43E; 25 m)	T average I-XII (ºC)	11.34±0.93	+ 2.15	+ 0.54	0.521***
	T average IV-X (ºC)	17.33±0.92	+ 2.15	+ 0.54	0.594***
	T average VI-VIII (ºC)	21.63±1.21	+ 3.25	+ 0.81	0.653***
	T maximum VII (ºC)	28.23±1.82	+ 3.65	+ 0.91	0.376***
	T minimum I (ºC)	-3.53±1.97	-	-	NS
	P I-XII (mm)	448.4±124.1	+ 200	+ 50	0.241***
	P VI-VIII (mm)	116.9±67.14	-	-	NS
	Huglin Index (ºC units)	2060±213	+ 440	+ 110	0.391***
	Winkler Index (ºC units)	1712±189	+ 445	+ 111	0.489***
	Cool Night Index (ºC)	12.30±1.53	+ 2.80	+ 0.70	0.265***
Craiova (44.31N; 23.86 E; 195m)	T average I-XII (ºC)	11.51±0.77	+ 1.70	+ 0.42	0.423***
	T average IV-X (ºC)	17.91±0.83	+ 1.50	+ 0.37	0.310***
	T average VI-VIII (ºC)	22.15±1.12	+ 2.40	+ 0.60	0.402***
	T maximum VII (ºC)	29.62±1.93	+ 3.50	+ 0.87	0.303***
	T minimum I (ºC)	-4.83±2.46	-	-	NS
	P I-XII (mm)	570.8±189.0	+ 195	+ 48	0.092*
	P VI-VIII (mm)	166.2±82.2	+ 50	+ 12.5	NS
	Huglin Index (ºC units)	2306±209	+ 435	+ 108	0.387***
	Winkler Index (ºC units)	1693±178	+ 350	+ 87	0.310***
	Cool Night Index (ºC)	11.55±1.91	-	-	NS

NS indicate trend that are not significant and *, **, and *** indicate significance at the 0.10, 0.05 and 0.01 levels, respectively.

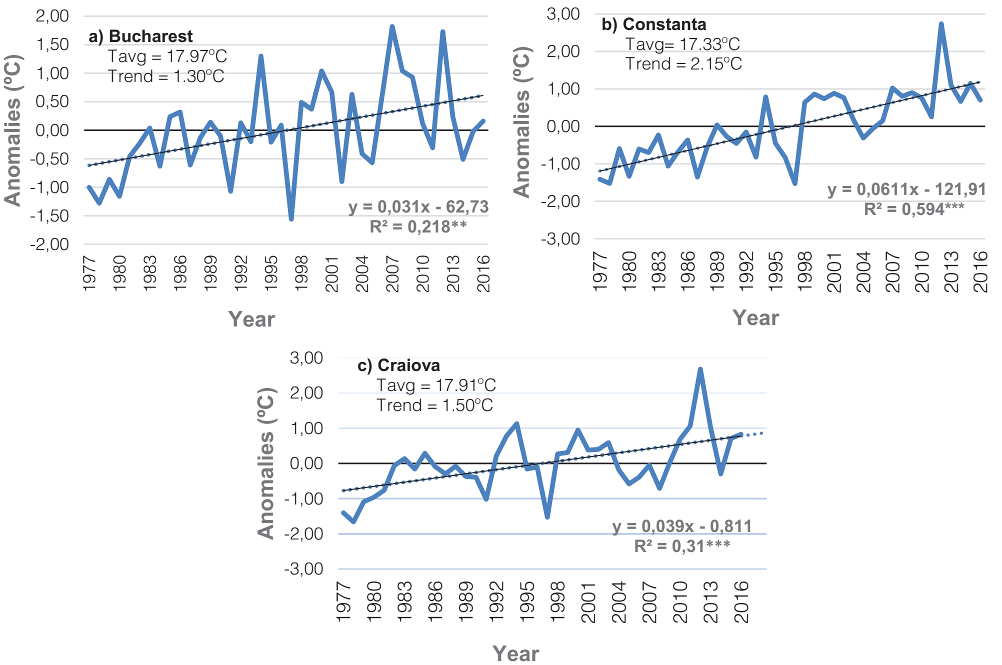


Figure 1. Registered growing season temperature anomalies in the period 1977-2016 for:
a) Bucharest; b) Constanta and c) Craiova; Tavg is the average growing temperature (April - October)

Grapevine behaviour. Climate warming has had different effects on the production of grapes (kg/vine), sugar accumulation (g/L) and titratable acidity (g/L H_2SO_4) of the grape, at Feteasca regala cultivar (Figures 2, 3, 4).

From Figure 2, it can be noted a high variability in grape yield from year to year and a slight reduction trend caused mainly by the minimum temperatures, harmful to vines during the dormant period (Tmin < -20ºC) in recent years (2005; 2010; 2012; 2015 and 2016). The frequency and intensity of these temperatures was discussed in a previous paper (Bucur and Dejeu, 2016).

From Figure 3, it is observed a highly significant trend of sugar content in berries. The statistical analysis of the data shows that sugar content depends on climatic suitability, which explains 65.5 % of the variance.

Regarding the titratable acidity of the grape must, it is found to its insignificant reduction over 19 years (Figure 4). However, in the last decade it was observed a reduction in titratable acidity of the must under 4 g/L, requiring acidification measures.

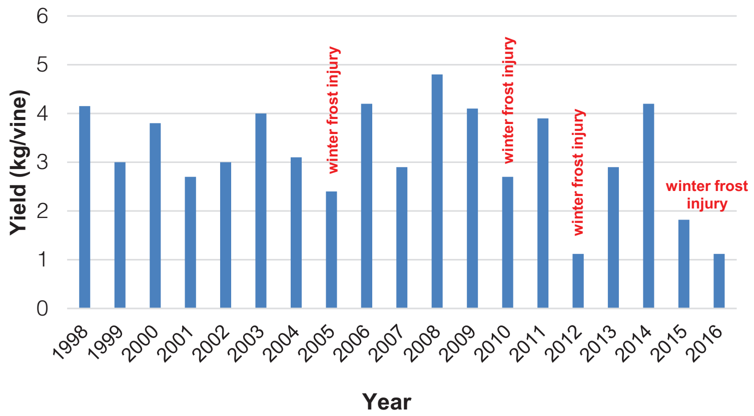


Figure 2. Evolution of grape yield (kg/vine) at Feteasca regala cultivar in the period 1998 - 2016

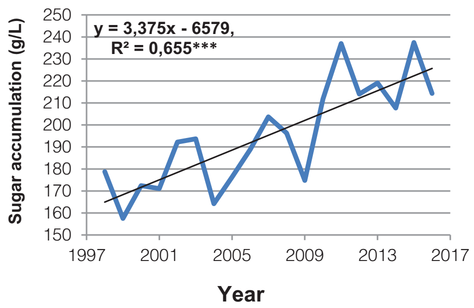


Figure 3. Evolution of sugar accumulation (g/L) at Feteasca regala cultivar in the period 1998-2016

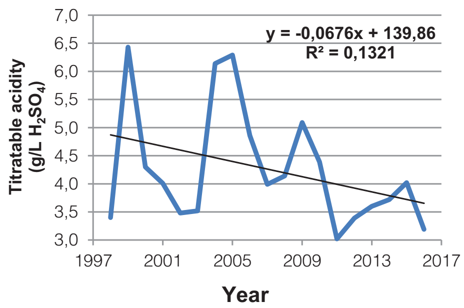


Figure 4. Evolution of titratable acidity (g/L H_2SO_4) of grape must at Feteasca regala cultivar in the period 1998-2016

CONCLUSIONS

During the last four decades, significant warming trends were observed in the three studied centers. This trend of temperature increase is almost certainly going to continue in a future warmer climate.

The results on Feteasca regala grapevine responses to climate change (high variability and trend to reduce grape yield, a highly significant increase of sugar content, reducing must acidity) are very important for the winegrowers because this is the most widely grown cultivar in Romania.

The results of this study provide the necessary information for viticultural zoning in the new conditions. Starting from the current situation of global warming and predictions for the future, in viticulture there should be implemented mitigation and adaptation measures.

REFERENCES

Botu I., Botu M., 2003. Analiză biostatistică şi design experimental în biologie şi agricultură. Editura Conphys, Râmnicu Vâlcea, 338 p.

Bucur Georgeta Mihaela, Dejeu L., 2016. Research on frost injury of new romanian grapevine cultivars in the winter 2014-2015. 5th International Conference „Agriculture for Life, Life for Agriculture", Agriculture and Agricultural Science Procedia 10, 233 – 237.

Burzo I., 2014. Modificările climatice și efectele asupra plantelor horticole. Editura Sitech, 7 - 41.

Cichi Daniela Doloris, 2006. Modificările termice din ecosistemul viticol. (Cauze, efecte asupra viţei de vie, studii). Editura Universitaria Craiova, 279 p.

Cotea V.V., Rotaru Liliana, Irimia L.M., Colibaba Lucia Cintia, Tudose Sandu-Ville S., 2008. The greenhouse effect on the viticultural ecoclimat in northern Moldavia, Romania. 31st World Congress of Vine and Wine, Verona, Italia.

Duchêne E., Schneider C., 2005. Grapevine and climatic changes: A glance and the situation in Alsace. Agronomy for Suistainable Developement, 25, 93, 99.

Huglin P., 1978. Nouveau mode d'évaluation des possibilités héliothermiques d'un milieu viticole. In: Symposium International sur l'Écologie de la Vigne, l, Constanta, Roumanie, 1978. Ministère de l'Agriculture et de l'Industrie Alimentaire, 89 - 98.

IPCC, 2014. Climate Change: impacts, adaptation and vulnerability.http://www.ipcc.ch/report/ar5/wg2/. Accessed on 20 January 2017.

Irimia L.M., Patriche C.V., Quenol H., Cotea V.V., 2015. Modificarea potențialului viticol al podgoriilor, ca efect al încălzirii climatului. Studiu de caz: Podgoria Cotnari. Hortus, Nr. 14, 187 - 192.

Jones G.V., White M.A., Cooper O.R., Storchmann K., 2005. Climate change and global wine quality, Climatic Change, 73(3), 319 - 343.

Keller M., 2015. The Science of Grapevines. Anatomy and Physiology. Elsevier, Academic Press, 509 p.

Palliotti A., Poni S., , Silvestroni O., 2015. La nuova viticoltura. Innovazioni tecniche per modelli produttivi efficienti e sostenibili. Edagricole-New Business Media, - Science, 544 p.

Ranca Aurora, Boloș P., Guluță E., 2008. Climate changes of the last 10 years in the Murfatlar vineyard and their influence on the behaviour of the specific grapevine varieties of this vineyard. 31st World Congress of Vine and Wine, Verona, Italy.

Rotaru Liliana, Colibaba Lucia Cintia, Prisăcaru Anca Irina, 2013. Studies of the behavioural tendencies of some grape varieties for white wines in Moldavian vineyards, under the influence of climatic changes. Lucrări Științifice USAMV „Ion Ionescu de la Brad" Iași, Seria Horticultură, vol.56, nr. 2, 303 - 308.

Tonietto J., Carbonneau A., 2004. A multicriteria climatic classification system for grape-growing regions worldwide. Agricultural and Forest Meteorology, 124(1/2), 81 - 97.

Winkler A.J., Cook A., Kliewer W.M., Lider I.A., 1974. General Viticulture. University of California Press, Berkeley, 740 p.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF FERMENTOR TYPE ON POLYPHENOL EXTRACTION IN RED WINES PRODUCED FROM CABERNET SAUVIGNON

George Adrian COJOCARU, Arina Oana ANTOCE

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Horticulture, Department of Bioengineering of Horti-Viticultural Systems,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author emails: arina.antoce@horticultura-bucuresti.ro; aantoce@yahoo.com

Abstract

Phenolic composition and colour of red wines produced from Cabernet Sauvignon grape variety were determined by means of specific spectrophotometric measurements in order to establish the influence of the maceration technique on the final wine quality. The results showed that the type of fermentor used during maceration-fermentation in red winemaking influence significantly the levels of anthocyanins, copigmentation colourless anthocyanins, polymeric pigments, flavones or total phenols. The age of vines had also specific influences which are thoroughly discussed in the paper. The highest values regarding total pigments (coloured, copigmented and colourless) were observed in wines from 3 years old vines, but they are less representative from the technological viewpoint. However, an increase in total phenols and total coloured pigments was observed when the 4 years old vines are compared with 5 years old vines. The type of fermentor used for the maceration-fermentation process is the most important for the total coloured pigments and total phenols extraction, these parameters ensuring structure and stable colour for wines. The highest levels of total coloured pigments were found in wines produced with horizontal fermentors with inner agitator, while the lowest values were obtained by using roto-fermentors, showing that the first are more suitable for producing well-coloured young wines. The results regarding colour intensity and hue revealed an increased colour intensity and lower hue when horizontal fermentors with inner agitator are used and lower colour intensity and higher hue when roto-fermentors are used. Although a higher hue values obtained when roto-fermentors are used means a higher oxidation, these fermentors also lead to a higher tannin extraction, being recommended when the resulted wines are intended for ageing.

Key words: *spectrophotometer, anthocyanin, pigments, maceration, red wine.*

INTRODUCTION

Colour, given by the anthocyanins free and combined with other polyphenols, is one of the most important intrinsic characteristic of red wines, being the first attribute evaluated during wine-tastings. Structure too, given by other specific polyphenols, is also important in red wine sensory appreciation and for the wine capacity to age. Certain technologies and treatments applied during vinification can influence the colour and the overall concentration of certain phenolic compounds in red wines. The oenologists are able to decide the appropriate technologies and treatments, in accordance with the desired final product characteristics (Cojocaru and Antoce, 2011; Antoce and Cojocaru, 2015; González-Neves et al., 2015; Gómez-Plaza et al., 2000; Busse-Valverde et al., 2011). Among the possible interventions, the effect of maceration-fermentation technique on wine colour and phenolic compounds is widely studied (Busse-Valverde et al., 2011; Casassa and Harbertson, 2014; González-Neves et al., 2013; Gambuti et al., 2009; Koyama et al., 2007; Gil-Munoz et al., 1999). Many important parameters of wines are influenced by maceration-fermentation process, including the polyphenol compounds extraction (Casassa and Harbertson, 2014; González-Neves et al., 2013; Gambuti et al., 2009; Koyama et al., 2007; Gil-Munoz et al., 1999; Jackson, 2008; Rakonczás et al., 2015). During maceration, along with anthocyanin and tannin extraction from grape skins potassium content is also increased, which leads to an increase of pH and changes in the total titratable acidity due to the potassium hydrogen tartrate precipitation (Drăghici and Râpeanu, 2011; Rakonczás et al., 2015; Peng et al., 1996), changes that can also affect the colour. Temperature, duration, homogenisation, aeration and enzyme addition during maceration are also important factors that have direct effect on extraction rate and final concentration in polyphenols or some other cellular constituents (Casassa et al., Harbertson, 2014; González-Neves et al., 2013; Gambuti et al., 2009; Koyama et al., 2007; Gil-Munoz et al., 1999; Jackson, 2008; Ribéreau-Gayon et al., 2006). Thus, for each style of wine, these parameters should be controlled when a certain maceration industrial technology is applied.

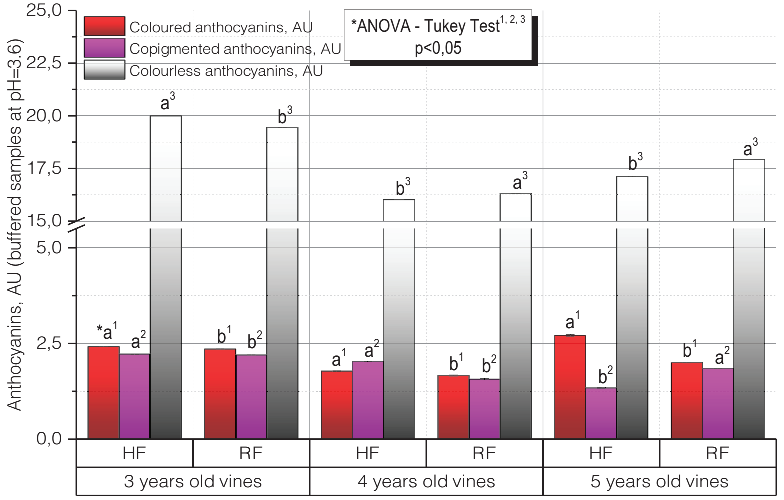
MATERIALS AND METHODS

The grapes for this study were harvested on October 2013 in Vrancea wine region from parcels containing Cabernet Sauvignon of 3, 4 and 5 years of age. Vinification of grapes involved minimal oenological intervention to reduce the influences induced during vinification in order to assess the effect of maceration techniques on phenolic composition and colour of wine. Grape batches were treated with 30 mg/kg with sulphur dioxide for antioxidant protection and then crushed and destemmed. The resulted grape mash batches from each parcel (with grapes from vines of 3 different ages) were transferred with progressive cavity pumps and each divided for maceration-fermentation in two type of fermentors: roto-fermentors (RF) and horizontal tank with inner agitator (HF). During the first day of maceration, all analysed tanks were treated with a preparation of pectolytic enzyme in dose of 1 g/q (Zymorouge G, AEB Spindal), for an enhanced extraction of tannins and pigments and better colour stabilisation. Cap management during maceration-fermentation was programmed in both types of tanks and achieved by 5 minutes of rotation for 3 times/day. The maceration and alcoholic fermentations were conducted at 24-28°C without inoculation (using the wild grape microflora) for 15 days, after which the marc batches were pressed with a horizontal press at 0.4 Bars. The resulted wines were then gravitationally clarified, racked and analysed to determine the parameters related to colour and phenolic composition. Six types of wines were obtained and used to compare the influence of maceration technique on phenolic composition. Wine samples were analysed in triplicate by assessing the main spectrophotometric parameters usually used to describe the colour and phenolic composition. The spectrophotometric methodology requires standardization of wine pH to 3.6 and filtration with PES membrane with 0.45 μm pore size. Each spectrophotometric measurement was performed with a UV - VIS double beam spectrophotometer Specord 250 from Analytik Jena AG using the software WinAspect version 2.2.7. In accordance to the parameter determined quartz or glass cuvettes were used. All the results were calculated to account for dilution and conventionally referred to the optical path of 10 mm and expressed in absorbance units (Antoce and Cojocaru, 2015). ***Monomeric anthocyanin*** concentration was determined as the difference in optical densities at 520 nm of the wine diluted to 5% and buffered to pH=3.6 in order to exclude copigmented anthocyanins (Boulton, 2001; Levengood and Boulton, 2004) and the same wine treated with sulphur dioxide to bleaching in order to exclude polymeric pigments and other resistant pigments (Jacobson, 2006; Eldridge and Liles, 1997). ***Copigmented anthocyanin*** concentration was determined as the difference in optical densities at 520 nm of the wine sample treated with an excess of acetaldehyde and of the same wine diluted down to 5% and buffered to pH=3.6. The excess of acetaldehyde reacts with the free SO_2 in wine, preventing any bleaching effect on the existent pigments, thus giving an estimation of total colour at 520 nm (Boulton, 2016, 2010 and 1996; Levengood and Boulton, 2004; Jacobson, 2006; Eldridge and Liles, 1997). ***Polymeric pigments and bleaching resistant pigments*** were determined as optical densitiy at 520 nm of the wine buffered at pH=3.6 and treated with an excess of SO_2. The effect of SO_2 is to bleach all monomeric and copigmented anthocyanins, but leave unaffected (coloured) the polymeric pigments and other non-bleachable derivatives of anthocyanins, which are formed through the addition of compounds such as pyruvate, acetaldehyde, hydroxycinnamates or vinylflavanols to the C4 and 5 hydroxyl positions of anthocyanins, generally known as pyranoanthocyanins (Somers, 1971; Boulton, 1996; Levengood and Boulton, 2004;

Harbertson and Spayd, 2006; Jacobson, 2006; Remy et al., 2000; Eldridge and Liles, 1997; Bakker et al., 1997; Bakker and Timberlake, 1997; Romero and Bakker, 1999; Mateus et al., 2001; Mateus and De Freitas, 2001; Cameira-dos-Santos et al., 1996; Fulcrand et al., 1996, 1997, 1998; Es-Safi et al., 1999; Benabdeljalil et al., 2000; Lu and Foo, 2001; Hayasaka and Asenstorfer, 2002; Schwarz et al., 2003). ***Colourless anthocyanins*** were determined as the difference in optical densities at 520 nm of the wine sample treated with excess of HCl and the same wine treated with excess of acetaldehyde. The addition of excess of HCl allows quantifying of total anthocyanins (coloured and colourless) by destroying the effect of concentration and the equilibrium formed at certain pH, while the acetaldehyde allows quantifying of total coloured anthocyanins (Jacobson, 2006; Eldridge and Liles, 1997). ***Flavone cofactors*** were determined at 365 nm, being the maximum of absorption wavelength for flavonoids involved in copigmentation phenomenon, especially quercetin and kaempferol (Eldridge and Liles, 1997; Merken and Beecher, 2000; Harbertson and Spayd, 2006). ***Total phenols*** were determined on a diluted wine sample (1/100) at 280 nm and corrected with a factor of 3.9 representing the non-polyphenol substances absorbing at the same 280 nm wavelength (Somers, 1998). ***Colour intensity*** and ***hue*** was calculated as the sum of absorbance determined at 420, 520, and 620 nm and, respectively, the ratio between absorbance at 420 nm and 520 nm (Ribéreau-Gayon et al., 2006; OIV, 2016) with mention that samples were buffered at pH=3.6 for standardization. The detailed spectrophotometric methodology is described in previous works (Boulton, 2016 and 1996; Levengood and Boulton, 2004; Jacobson, 2006; Eldridge and Liles, 1997). The Origin 10.0 software program was used for data processing. Analysis of variance (ANOVA) was applied to the results with posthoc Tukey Test for the comparison of means, at 0.05 significance level.

RESULTS AND DISCUSSIONS

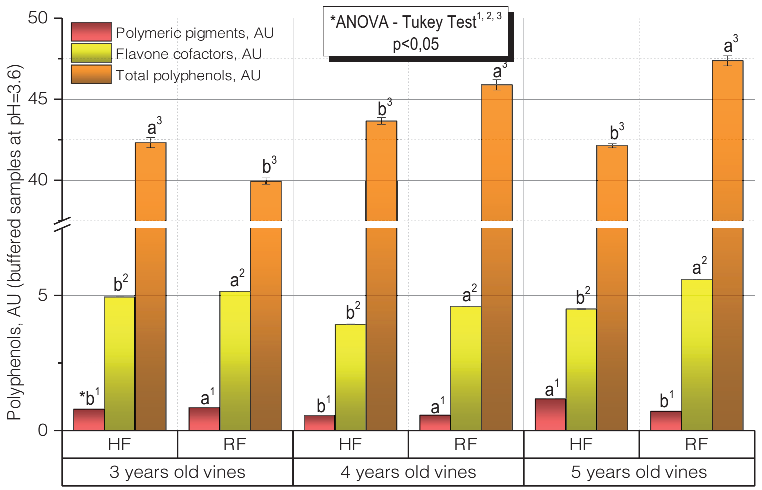
The results presented in figure 1 show the variation of coloured, copigmented and colourless anthocyanins in Cabernet Sauvignon wines produced with grapes coming from vine plantations of 3, 4 and 5 years of age, each macerated in two types of tanks. As expected, we can observe slight differences in coloured anthocyanin between vines with different ages, the highest value being observed in wines produced from 5 year old vines using the horizontal fermentor with inner agitator and the lowest being observed in wines produced from 4 year old vines using the roto-fermentor. Generally, when the horizontal fermentor with inner agitator was used, slightly higher content in coloured monomeric anthocyanin was observed in wine samples, irrespective to vine age, compared to wines produced with roto-fermentors. However, regarding the copigmented anthocyanin, the highest content was observed in wines produced from 3 year old vines, while the lowest content was observed in wines produced from 5 year old vines. This case is similar for monomeric coloured anthocyanin regarding the type of fermentor used with the exception of the copigmented anthocyanins observed in the wines produced from 5 years old vines. The lower values of copigmented anthocyanins obtained in the horizontal fermentor with inner agitator are well explained by the larger values of polymeric pigments shown in figure 2. Generally, aside of the enhancement in absorbance it is believed that the copigmented anthocyanins are do not affect the rate of polymerization reactions during wine ageing (Bimpilas et al., 2016; Boulton, 2001). A possible pathway of polymeric pigments formation was suggested by previous works, considering that the hydrophobic stacking interaction between anthocyanin chromophores and the so-called copigments, could be the first step in the formation of a covalent bond between the anthocyanin and its copigment (Brouillard and Dangles, 1994). However, the polymerization reactions during ageing are involved in formation of a more stable coloured pigments (Jackson, 2008; Somers, 1971). Between colourless hydrated form of anthocyanins and coloured flavylium cation an equilibrium forms based mainly on the wine pH, determined not only by the thermodynamic constants of anthocyanin molecules, but also by the concentration, solvent and temperature. The colourless hydrated form of anthocyan can act as nucleophile, being involved in condensation reactions with flavan-3-ols as electrophiles in wines, leading to larger red pigments of flavanol-anthocyanin ($F - A^+$) (Cheynier et al., 2000; Brouillard and Delaporte, 1977).



***ANOVA results:** the population means for **age of vines** are significantly different; the population means for **type of fermentor** are significantly different; the interaction between **age of vines** and **type of fermentor** is significant.

**HF - Horizontal fermentor with inner agitator; RF - Roto-fermentor;

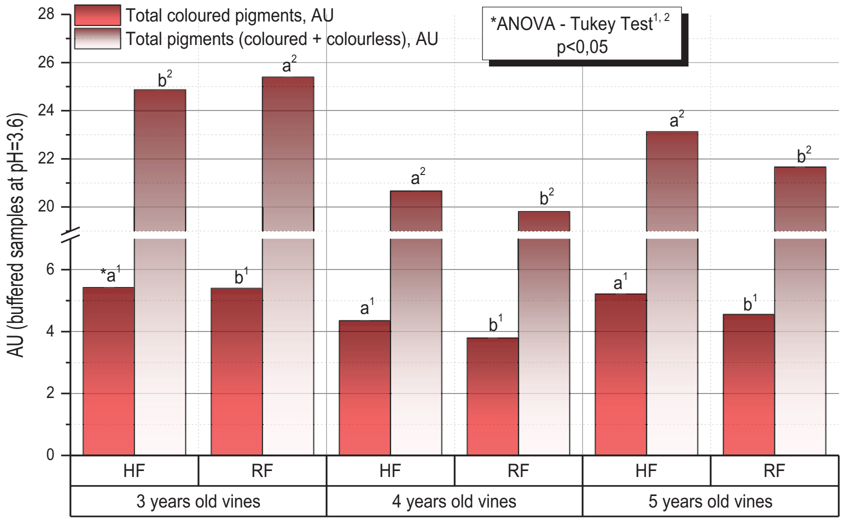
Figure 1. Variation of anthocyanin content in Cabernet Sauvignon wines produced with two types of maceration techniques from grapes with different age of vine plantations



***ANOVA results:** the population means for **age of vines** are significantly different; the population means for **type of fermentor** are significantly different; the interaction between **age of vines** and **type of fermentor** is significant.

**HF - Horizontal fermentor with inner agitator; RF - Roto-fermentor;

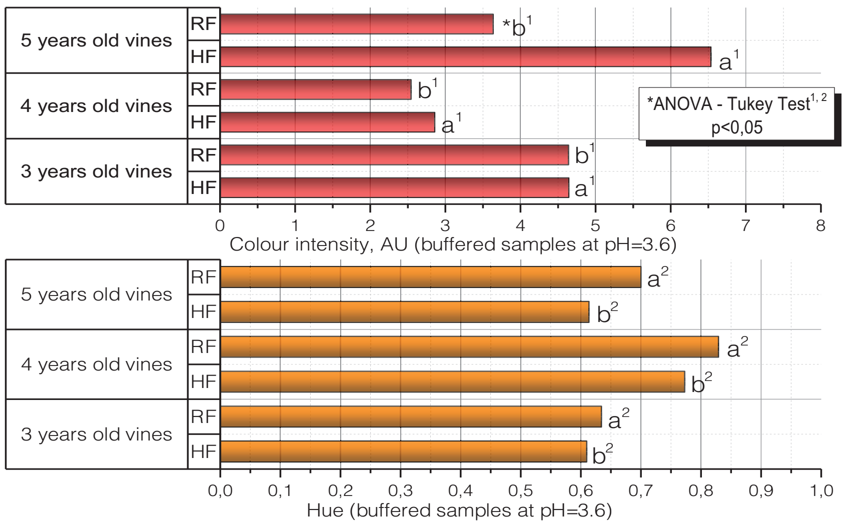
Figure 2. Variation of certain polyphenols content in Cabernet Sauvignon wines produced with two types of maceration techniques from grapes with different age of vine plantations



***ANOVA results:** the population means for **age of vines** are significantly different; the population means for **type of fermentor** are significantly different; the interaction between **age of vines** and **type of fermentor** is significant.

**HF - Horizontal fermentor with inner agitator; RF - Roto-fermentor;

Figure 3. Variation of total coloured pigments and total pigments (coloured + colourless) in Cabernet Sauvignon wines produced with two types of maceration techniques from grapes with different age of vine plantations



***ANOVA results:** the population means for **age of vines** are significantly different; the population means for **type of fermentor** are significantly different; the interaction between **age of vines** and **type of fermentor** is significant.

**HF - Horizontal fermentor with inner agitator; RF - Roto-fermentor;

Figure 4. Variation of colour intensity and hue in Cabernet Sauvignon wines produced with two types of maceration techniques from grapes with different age vine of plantations

On the other hand, the red flavylium cation (coloured anthocyanins) can act as electrophile, being involved in condensation reactions with flavan-3-ols as nucleophile in wines, leading to larger red pigments of anthocyanin-flavanol (A^+ - F) (Cheynier et al., 2000; Remy et al., 2000).

Our results regarding colourless (hydrated) form of anthocyanins presented in figure 1 show greater values for 3 years old vines, which explains why the total content of anthocyanins (coloured, copigmented and colourless) presented in figure 3 is greater in wines produced from 3 years old vines. This phenomenon is probably due to the fact that being in the first year of production the 3 years old vines have low yields, thus accumulating more anthocyanin per grape. However, the effect of concentration, standardization of pH to 3.6 and alcoholic concentration affects this specific equilibrium of anthocyanin forms, leading to the results presented in figure 1. The polymeric pigments, flavone cofactor and total phenolic compounds are presented in figure 2.

The level of total phenols in the grapes and wines is influenced significantly by the age of vines, being greater in wines produced from 4 and 5 years old vines. Due to the high concentration of tannins (figure 2) in wines produced from 5 years old vines, in the case of a higher concentration of coloured anthocyanins (as flavylium cations) – the wines produced in horizontal fermentor (figure 1), the formation of polymeric pigments of anthocyanin-flavanol (A^+ - F) type seem to be favoured (figure 2). However, the other mechanism for the production of flavanol-anthocyanin type of polymeric pigments (F - A^+) seems to be slower in the wines from 5 years old vines. This was not the case in the wine samples produced from 3 years old vines, where we have found higher concentrations of colourless hydrated anthocyanin (figure 1), but similar values for polymeric pigments with wines produced for 5 years old vines (figure 2). Thus, based on a single study, the observed results on polymeric pigments formation are not very conclusive. Another study have already shown that certain winemaking parameters (increased temperature during storage or pH = 3.8) can lead to formation of anthocyanin-flavanol (A^+ - F) polymeric pigments (Fulcrand et al., 2006).

Generally, observations on wine samples produced from 4 and 5 years old vines, showed that the roto-fermentors tend to extract more phenols (tannins) than the horizontal fermentors with inner agitator (figure 2).

Also, the results regarding total coloured pigments presented in figure 3, are not well correlated with colour intensity of wine samples (figure 4). However, when horizontal fermentors were used, greater values were observed in both types of analyses irrespective of the age of vines and, correspondingly, lower values when roto-fermentors were used. Total pigments (coloured and colourless) are generally higher when horizontal fermentors with inner agitator were used (figure 3). Only in wines produced from 3 years old vines we have observed higher concentrations of total pigments (coloured and colourless) with the use of roto-fermentor. The results regarding hue of colour in figure 4, revealed that wines produced with roto-fermentors are more oxidized, with more yellow tones than wines produced with horizontal fermentors with inner agitator.

CONCLUSIONS

Polyphenol extraction in wines is influenced significantly by the type of fermentor used, but the age of vines, especially when they are very young, can also have a major effect on wine quality. Different equilibrium between anthocyanin forms (coloured, copigmented and colourless) could be observed in relation with age of vines and also the type of fermentor used during the process of maceration. Generally, the content of total pigment (coloured, copigmented and colourless) was higher in wine samples produced from 3 years old vines, possibly due to a low yield and higher anthocyanin accumulation/grape, but this case is less representative from the technological point of view. An increasing trend was observed in the case of total coloured pigments in the case of the 4 and 5 years old vines, with similar behaviours regarding the colour intensity, even though not correlated to the vine age. The type of fermentor used during maceration-fermentation has a significant effect on extraction of anthocyanins and tannins and implicitly big impact the structure and colour in red wines. An increased extraction of total phenols and a decreased total coloured pigments was observed in wines produced with roto-fermentors, and an opposite effect was observed in wines produced with horizontal fermentors with inner agitator. Colour intensity has a similar behaviour with total coloured pigments, more intense colour being observed when wines are produced with horizontal fermentors. The analyses regarding hue of colour showed that roto-fermentors produced wines with increased values, which suggest an advanced oxidation compared to the wines from horizontal fermentors with inner agitator. However, although the level of total coloured pigments and colour intensity are founded in greatest concentrations when wines are produced with horizontal fermentors, and also, the hue values are lower than in wines from roto-fermentors, we recommend this later type of fermentor to be used when wines with better polypehnolic structure meant for aging are desired. The horizontal fermentors, with a higher extraction of anthocyanins are more suitable for the production of intensely coloured wines designed to be commercialised young.

REFERENCES

Antoce A. O., Cojocaru G. A., 2015. Technological approaches to the vinification of Dornfelder grape variety. 38th World Congress of Vine and Wine (Part 1), BIO Web Conferences, Vol. 5, 02009.

Bakker J., Bridle P., Honda T., Kuwano H., Saito N., Terahara N., Timberlake C. F., 1997. Identification of an anthocyanin occurring in some red wines. Phytochemistry, Vol. 44, No. 7, 1375–1382.

Bakker J., Timberlake C. F., 1997. Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. Journal of Agricultural and Food Chemistry, Vol. 45, No. 1, 35–43.

Bakker J, Preston N.W., Timberlake C. F., 1986. The determination of anthocyanins in ageing red wine: Comparison of HPLC and Spectral methods. American Journal of Enology and Viticulture, Volume 37, No. 1, 121-126.

Benabdeljalil C., Cheynier V., Fulcrand H., Hakiki A., Mosaddak M., Moutounet, M., 2000. Evidence of new pigments resulting from reaction between anthocyanins and yeast metabolites. Sciences des Aliments, Vol. 20, No. 2, 203–220.

Bimpilas A., Panagopoulou M., Tsimogiannis D., Oreopoulou V., 2016. Anthocyanin copigmentation and color of wine: The effect of naturally obtained hydroxycinnamic acids as cofactors. Food Chemistry Vol. 197, Part A, 39-46.

Boulton R. B., 2016. The copigmentation assay for red wines. University of California, Davis, Department of Viticulture and Enology website: http://boulton.ucdavis.edu/copig.htm, accessed at 15th December 2017.

Boulton R., 2001. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. American Journal of Enology and Viticulture, vol. 52, no. 2, 67-87.

Boulton R. B., 1996. Methods for the assessment of copigmentation in red wines. 47th Annual Meeting of the American Society for Enology and Viticulture, Reno, NV.

Brouillard R., Dangles O., 1994. Anthocyanin molecular interactions: the first step in the formation of new pigments during wine aging? Food Chemistry Vol. 51, Issue 4, 365-371.

Brouillard R., Delaporte B., 1977. Chemistry of anthocyanin pigments. 2. [1]Kinetic and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin 3-glucoside. Journal of the American Chemical Society, Vol. 99, Issue 26, 8461-8468.

Busse-Valverde N, Gómez-Plaza E., López-Roca J. M., Gil-Muñoz R., Bautista-Ortín A. B., 2011. The extraction of anthocyanins and proanthocyanidins from grapes to wine during fermentative maceration is affected by the enological technique. Journal of Agriculture and Food Chemistry Vol. 59, No. 10, 5450-5455

Cameira-dos-Santos P. J., Brillouet J. M., Cheynier V., Moutounet M., 1996. Detection and partial characterisation of new anthocyanin-derived pigments in wine. Journal of the Science of Food and Agriculture, Vol. 70, No. 2, 204–208.

Casassa L. F., Harbertson J, 2014. Extraction, Evolution, and Sensory Impact of Phenolic Compounds during Red Wine Maceration. Annual Review of Food Science and Technology, Vol. 5, No. 1, 83-109.

Cheynier V., Remy S., Fulcrand H., 2000. Mecanism of anthocyanin and tannin changes during winemaking and aging. Proceedings of the American Society for Enology and Viticulture, 50th Anniversary Annual Meeting, Seattle, Washington, USA, 337-344.

Cojocaru G. A., Antoce A. O., 2011., Color Changes Induced by Fining Treatments in Red Winemaking. Lucrări ştiinţifice U.Ş.A.M.V.B., Seria B, Vol. LV, 2011, Section Viticulture & Oenology, Congres CD, ISSN 1222-5312, 496-500.

Drăghici L., Râpeanu G., 2011. Evolution of polyphenols during the maceration of the red grapes. Journal of Agroalimentary Processes and Technologies, Vol. 17, No. 2, 169-172.

Eldridge D., Liles J., 1997 (revised in 2002 by: Bechard Andrea and Ritchie Gerry). Spectral Measures for estimating Wine Color and Phenolics. Napa Valley College Viticulture and Enology Department and Napa Valley Vintners Association Teaching Winery, Wine and Must Analyses Manual, Standard Operating Procedure.

Es-Safi N. E., Guernevé C. L., Labarbe B., Fulcrand H., Cheynier V., Moutounet M., 1999. Structure of a new xanthylium salt derivative. Tetrahedron Letters, Vol. 40, No. 32, 5869–5872.

Fulcrand H., Duenas M., Salas E., Cheynier V., 2006. Phenolic Reactions during Winemaking and Aging. American Journal of Enology and Viticulture, Vol. 57, Issue 3, 289–297.

Fulcrand H., Benabdeljalil C., Rigaud J., Cheynier V., Moutounet M., 1998. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. Phytochemistry, Vol. 47, No. 7, 1401–1407.

Fulcrand H., Cheynier V., Oszmianski J., Moutounet M., 1997. An oxidized tartaric acid residue as a new bridge potentially competing with acetaldehyde in flavan-3-ol condensation. Phytochemistry, Vol. 46, No. 2, 223–227.

Fulcrand H., Cameira-dos-Santos P. J., Sarni-Manchado P., Cheynier V., Favre-Bonvin J., 1996. Structure of new anthocyanin-derived wine pigments. Journal of the Chemical Society, No. 7, 735–739.

Gambuti A, Capuano R, Lecce L., Fragasso M. G., Moio L., 2009. Extraction of phenolic compounds from 'Aglianico' and 'Uva di Troia' grape skins and seeds in model solutions: Influence of ethanol and maceration time. Vitis, Vol. 48, No. 4, 193–200.

Gil-Munoz R., Gómez-Plaza E., Martínez A., López-Roca J. M., 1999. Evolution of Phenolic Compounds during Wine Fermentation and Post-fermentation: Influence of Grape Temperature. Journal of Food Composition and Analysis, Vol. 12, 259-272.

Gómez-Plaza E., Gil-Muñoz R., López-Roca J. M., Martínez A., 2000. Color and phenolic compounds of a young red wine. Influence of wine-making techniques, storage temperature, and length of storage time. Journal of Agriculture and Food Chemistry, Vol. 48, No. 3, 736-741.

González-Neves G., Favre G., Gil G., Ferrer M., Charamelo D., 2015. Effect of cold pre-fermentative maceration on the color and composition of young red wines cv. Tannat. Journal of Food Science and Technology, Vol. 52, No. 6, 3449-3457.

González-Neves G., Gil G., Favre G., Baldi C., Hernández N., Traverso S., 2013. Influence of Winemaking Procedure and Grape Variety on the Colour and Composition of Young Red Wines. South African Journal of Enology and Viticulture, Vol. 34, No. 1, 138-146.

Harbertson J. F., Spayd S., 2006. Measuring Phenolics in the Winery. American Journal of Enology and Viticulture Vol. 57, No. 3, 280-288.

Hayasaka Y., Asenstorfer R. E., 2002. Screening for potential pigments derived from anthocyanins in red wine using nanoelectrospray tandem mass spectrometry," Journal of Agricultural and Food Chemistry, Vol. 50, No. 4, 756–761.

Jacobson J. L., 2006. Introduction to Wine Laboratory Practices and Procedures, Springer Science + Business Media, Inc.

Jackson R. S., 2008. Wine Science: Principles and Applications 3rd Edition. Academic Press is an imprint of Elsevier.

Koyama K., Goto-Yamamoto N., Hashizume K., 2007. Influence of Maceration Temperature in Red Wine Vinification on Extraction of Phenolics from Berry Skins and Seeds of Grape (*Vitis vinifera*). Bioscience, Biotechnology, and Biochemistry, Vol. 71, No. 4, 958-965.

Levengood J., Boulton R., 2004. The variation in the color due to copigmentation in young Cabernet Sauvignon wines. *In* Red Wine Color: Exploring the Mysteries. A.L. Waterhouse and J.A. Kennedy (Eds.), 35-52. ACS Symp. Ser. 886. Am. Chemical Society, Washington, DC.

Lu Y., Foo L. Y., 2001. Unusual anthocyanin reaction with acetone leading to pyranoanthocyanin formation, Tetrahedron Letters, Vol. 42, No. 7, 1371–1373.

Mateus N, Silva A. M. S., Vercauteren J., De Freitas V., 2001. Occurrence of anthocyanin-derived pigments in red wines. Journal of Agricultural and Food Chemistry, Vol. 49, No. 10, 4836–4840.

Mateus N., De Freitas V., 2001. Evolution and stability of anthocyanin-derived pigments during port wine aging. Journal of Agricultural and Food Chemistry, Vol. 49, No. 11, 5217–5222.

Merken H. M., Beecher G. R., 2000. Measurement of food flavonoids by high-performance liquid chromatography: a review. Journal of Agriculture and Food Cemsitry, 48, 577.

OIV, 2016. Chromatic Characteristics. Section 2 – Physical analysis in Compendium of International Methods of Analysis, OIV-MA-AS2-07B.

Peng Z., Waters E. J., Pocock K. F., Williams P. J., 1996. Red wine bottle deposits, II: Cold stabilisation is an effective procedure to prevent deposit formation. Australian Journal of Grape and Wine Research, Vol. 2, Issue 1, 1-5.

Rakonczás N., Andrási D., Murányi Z., 2015. Maceration affect mineral composition and pH of wines. International Journal of Horticultural Science, Vol. 21 No. 3–4, 25–29.

Remy S., Fulcrand H., Labarbe B., Cheynier V., Moutounet M., 2000. First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions. Journal of the Science of Food and Agriculture Vol. 80, No. 6, 745–751.

Ribéreau-Gayon P., Glories Y., Maujean A., Dubordieu D., 2006. Handbook of Enology (vol. 2). The Chemistry of Wine, Stabilization and Treatments 2nd Ed., John Wiley & Sons Ltd.

Romero C., Bakker J., 1999. Interactions between grape anthocyanins and pyruvic acid, with effect of pH and acid concentration on anthocyanin composition and color in model solutions. Journal of Agricultural and Food Chemistry, Vol. 47, No. 8, 3130–3139.

Schwarz M., Jerz G., Winterhalter P., 2003. Isolation and structure of Pinotin A, a new anthocyanin derivative from Pinotage wine. Vitis, Vol. 42, No. 2, 105–106.

Somers T. C., 1971. The polymeric nature of wine pigments. Phytochemistry Vol. 10, No. 9, 2175–2186.

Somers T. C., 1998. The Wine Spectrum. An Approach towards Objective Definition of Wine Quality. Hyde Park Press, Adelaide: Winetitles.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PHENOLOGICAL CHANGES OF SHOOT CARBOHYDRATES AND PLANT GROWTH CHARACTERISTICS IN *VITIS LABRUSCA* L. GRAPE

Bülent KÖSE[1], Hüseyin ÇELIK[2]

[1]Ondokuz Mayıs University, Samsun Vocational School,
Plant and Animal Production Program, Samsun, Turkey
[2]Ondokuz Mayıs University, Agricultural Faculty,
Department of Horticulture, Samsun, Turkey

Corresponding author email: bulentk@omu.edu.tr

Abstract

Stored carbohydrates are used to supply energy in the next season for shoot growing, flowering and ripening in grapevines. Carbohydrates are transfers from storage organs to growth areas following the bud burst in spring. The present study was carried out to determine the effect of different rootstocks on growth characteristics and phenological changes of shoot carbohydrates in 10 years-old Vitis labrusca L. grapes. The grapevines are grafted on 140Ru, 5BB and 5C rootstocks. In the experiment; changes of sugar, starch and total carbohydrate contents in annual shoots were investigated in different phenological stages. While the highest sugar and total carbohydrate content of shoots were determined in veraison period on 5BB grafted grapevines, starch content was high in the harvest on 140Ru. While the highest mean and total leaf areas were calculated in grapevines grafted on 140Ru rootstock, shoot length and diameter was the highest on 5BB rootstock. Leaf chlorophyll content was the highest in blooming period on 5C grafted grapevines.The heavier fruit clusters, berries and total yield per vine were obtained on 5BB grafted vines. In this study, 5BB rootstock was found to be favorable in terms of shoot carbohydrate content and growth characteristics for Vitis labrusca L. grape.

***Key words**: carbohydrate, growth, rootstocks, Vitis labrusca L.*

INTRODUCTION

Rootstocks have significant effects on the growth; shoot development, yield, nutrient uptake and resistance to phylloxera of grapevines (Verma et al., 2010; Jogaiah et al., 2013; Rafaat et al., 2013).
Rootstocks also play essential roles in dry matter partition of root, stem, shoot and fruit (Loescher et al., 1990; Somkuwar, 2012). Therefore, grapevine growth and development are closely related to cultural practices performed throughout the growing season.
Rootstocks influence sugar and starch reserves of grapevines (Smith, 2004; Jogaiah et al., 2013). For instance, in ‘Merzifon Karasi’ grapes, the highest root carbohydrate content was observed in grapevines grafted on 5C rootstock and the highest stem and shoot soluble carbohydrate contents were determined in grapevines grafted on 110R and 5C rootstocks (Köse et al., 2014).
Carbohydrates are responsible for the shoot development, root and stem diameter growth, flower bud development, graft union and fruit set in various plant species (Caspari et al., 1998; Göktürk et al., 2005; Loescher et al., 1990). Carbohydrates throughout the spring season started to be used rapidly with the initiation of growth.
During to development of grapevine, shoot development slows down especially during the fruit set period and carbohydrates promptly start to accumulate in shoots (Weaver, 1976). Carbohydrate accumulation then slows down with the recess in fruit size and shoot growth almost ceases at the end of summer (Van der Zijpp and Creagh, 2011).
In ‘Shiraz’ grapes, carbohydrate concentration decreased in all tissues in veraison period and increased again in the harvest period (Smith et al., 2009).
Carbohydrates transport following the bud burst in spring to support shoot development and initiation of flowering varies mostly based

on species, fruit load and climate conditions (Bates et al., 2002; Holzapfel and Smith, 2012; Zufferey et al., 2012). Soluble sugars in upper-soil organs have significant contributions to the total carbohydrate content (Holzapfel and Smith, 2012; Zufferey et al., 2012). While starch concentration decreases in the dormancy period, sugar concentration increases.

The highest starch levels in shoot, stem and root are commonly observed at the end of growing season (Winkler and Williams, 1945). Such a chance is related to winter-resistance and increased conversion of starch into sugar against cold damage (Hamman et al., 1996).

The experiment was conducted to investigate the growth characteristics and phenological changes of shoot carbohydrate accumulation in *Vitis labrusca* L. grape grafted on different rootstocks.

MATERIALS AND METHODS

The research was conducted in the experimental vineyard of Ondokuz Mayıs University, Turkey. The vineyard located at 41°21'52 N latitude and 36°11'29 E longitude with an altitude of 195 m and a distance of about 2.8 km from the Black Sea coast of Turkey. The experiment was conducted from December 2012 to October 2013, in vines of the 10-years *Vitis labrusca* L. grapes were grafted on 140Ru, 5BB and 5C rootstocks.

The grapes have a foxy flavor, thick slip skins and a distinct aroma, and are consumed as table grapes, in marmalades and pickles, or as juice according to the local needs of all coastal areas of the Black Sea Region of Turkey. Short pruned grapevines were trained onto a high double cordon with 3x1.5 m spacing. Grapevines were not irrigated, not fertilized and the only supplementary water received from rainfall.

Shoot sugar, starch and total carbohydrate contents were determined at modified Eichhorn-Lorenz phenological stages (Coombe, 1995). Shoot length (cm), diameter (mm), mean and total leaf area (cm^2) were measured from pre-bloom to harvest.

Shoot length and diameter was measured at three selected shoots of each grapevine. Shoot diameter was measured on between two and three nodes of shoots with digital compass. Leaf areas were calculated non-destructively $[-1.41 + 0.527*(W^2) + 0.254*(L^2)]$ according to Elsner and Jubb (1988). Leaf width (W) and leaf length (L) were used to calculate leaf areas. The analysis of TSSC (Total Soluble Solids Content) readings was performed by a digital refractometer (Atago Co. I. Japan).

Grape cluster and berries were weighted by digital scale (0.1 g accuracy) at harvest.

Titratable acidity was used by titration with 0.1 N NaOH at pH 8.3, using phenolphthalein as indicator.

Air temperature, relative humidity and precipitation data were obtained from Turkish State Meteorological Service, Samsun Regional Office. Monthly average temperature, relative humidity and precipitation were calculated from the daily records (Figures 1 and 2).

Experiment area has clay soil type. Physical characteristics of experimental vineyard are provided in Table 1.

Table 1. Physicochemical properties and nutrient contents of the vineyard soil

Soil Properties		Nutrient Element Contents	
Clay %	62,12	Toplam N, %	0.212
Silt %	20,09	P, mg kg^{-1}	42.77
Sand%	17,12	K, me 100 g^{-1}	1.07
Texture class	SC*	Ca, me 100 g^{-1}	28.39
pH, sat. Ext.	7.1	Mg, me 100 g^{-1}	8.96
EC, (dS m^{-1})	0.69	Fe, mg kg^{-1}	12.02
OM, %	4.33	Mn, mg kg^{-1}	23.63
Lime ($CaCO_3$), %	0.85	Zn, mg kg^{-1}	1.82
CEC, meq $100g^{-1}$	30.05	Cu, mg kg^{-1}	1.11

* Soil-clay

Chlorophyll analysis

Chlorophyll a (Ch_a) and chlorophyll b (Ch_b) concentrations were determined using the method described by Lichtenthaler and Wellburn (1983). Fresh leaves (0.1 g) were placed into 8 ml of 80% acetone, and filtered through Whatman No. 2 filter paper.

After absorbance was measured in a UV-visible spectrophotometer (Pharmacia LKB-Novaspec II model spectrophotometer, UK) at 646, 663 and 470 nm, Ch_a and Ch_b (mg/g FW) were calculated according to the following equations:

Chlorophyll a (Ch_a) = 12.25 A_{663}– 2.798 A_{646}

Chlorophyll b (Ch_b) = 21.5 A_{646}– 5.1 A_{663}

Total Chlorophyll = Chlorophyll a (Ch_a) + Chlorophyll b (Ch_b)

Carbohydrate analysis

The samples taken between the 2nd and 3rd nodes of the shoots during the bud burst (E-L 02), pre-blooming (E-L 15), full blooming (E-L 23), veraison (E-L 35), harvest (E-L 38), after harvest (E-L 43) and dormancy (E-L 47) periods were dried in an oven at 60 ^{o}C for 48 hours.

Plant tissue samples were pulverized in a mill (IKA, Staufen, Germany) with a 40-mesh screen for analysis and 200 mg of dust was used for the extraction.

Soluble sugars were extracted twice with 8 ml of 80% ethanol at 60°C for 30 minutes (Candolfi and Koblet, 1990).

Soluble sugar content was determined with the anthrone method as described by Scott and Melvin (1953).

Starch was extracted twice with 8 ml of 1 M perchloric acid, one hour each time at 60°C and measured by the same method.

Absorbance readings were made at 620 nm with a spectrophotometer.

Glucose was used as a standard for the analysis of soluble sugars and starch.

Statistical analysis

Complete randomized block design with 3 replications were used in the study and the mean measurements were calculated. Growth characteristics and carbohydrate content were measured in four grafted vines of each replication per rootstock. The data were analyzed by two-way analysis of variance (ANOVA) to test main effects and interactions between for phenological stages and rootstocks. Data analysis was performed using SPSS 16.0 for Windows. Results studied were presented as means and a pooled standard error of mean (SEM). Differences among means were detected using Duncan's multiple range tests at significance levels of (p <0.01).

RESULTS AND DISCUSSION

Mean monthly temperature (°C), relative humidity (%) and precipitation (mm) values belonging to the long term and experiment year for phenological stages were are in Figures 1 and 2. According to long term data, mean temperatures in bud burst period were calculated as 11.4 ^{o}C. In blooming, mean temperatures were calculated as 20.3 ^{o}C.

The highest average temperatures were measured during veraison to harvest. Thereafter, temperatures decreased.

In contrast to average temperatures, relative humidity did not vary substantially throughout the season. In bud burst period, the monthly precipitation was determined as 56.5 mm. According to long term climate data, total precipitation was calculated as 274.7 mm from bud burst to harvest period. Minimum precipitation was measured in the veraison and harvest period.

The month precipitation was determined as 32.0 mm in June and 40.1 mm in July. During the growth season, total precipitation was calculated as 223 mm from bud burst to harvest period (Figure 1).

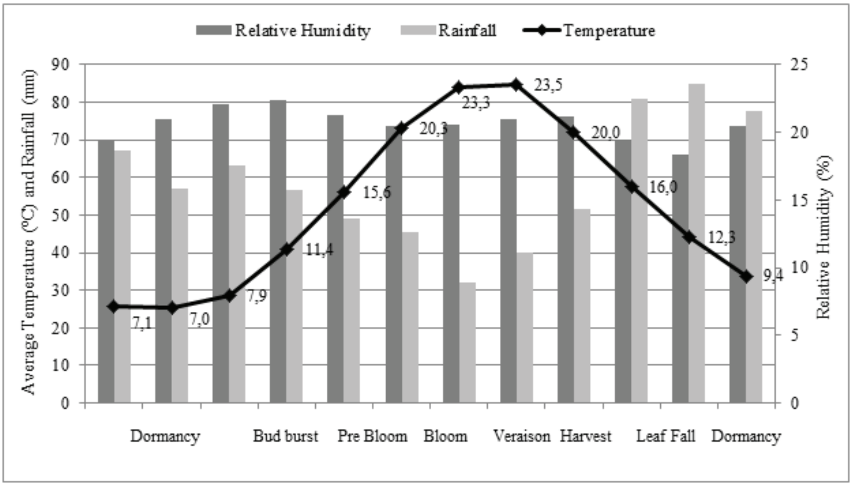


Figure 1. Changes of monthly mean temperature (°C), precipitation (mm) and relative humidity (%) throughout the growth seasons according to long term data (1960 to 2014).

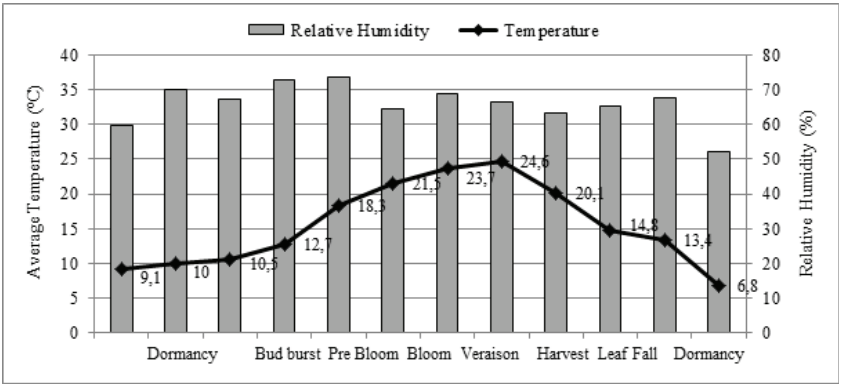


Figure 2. Changes of monthly average temperature (°C) and relative humidity (%) from bud burst to dormancy period in the experiment year.

In the experimental season, mean temperatures in the bud burst period were calculated as 10.5 °C. In blooming, mean temperatures were calculated as 21.5 °C. The highest average temperatures were measured during veraison as 24.6 °C. After the veraison stage, temperatures decreased gradually. Relative humidity was calculated respectively as 64.6% in blooming and 63.2 % in harvest (Figure 2).

Changes of sugar, starch and carbohydrates of annual shoots

Phenological changes in sugar, starch and total carbohydrate contents of annual shoots of *Vitis labrusca* L. grapes grafted on 140 Ru, 5BB and 5C rootstocks are presented in Figure 3, 4 and 5. Significant differences were observed in sugar contents of the annual shoots between rootstocks and phenological periods ($p<0.01$). Sugar content of shoots was the highest in veraison stage. The lowest sugar level was obtained at the blooming and harvest stages. The highest sugar contents were determined in grapevines grafted on 5BB rootstock in pre-blooming (18.03 mg L^{-1}) and veraison stages (24.32 mg L^{-1}). The lowest sugar contents were measured in grapevines grafted on 5C rootstock during the harvest (4.79 mg L^{-1}) and leaf fall period (4.90 mg L^{-1}) (Figure 3).

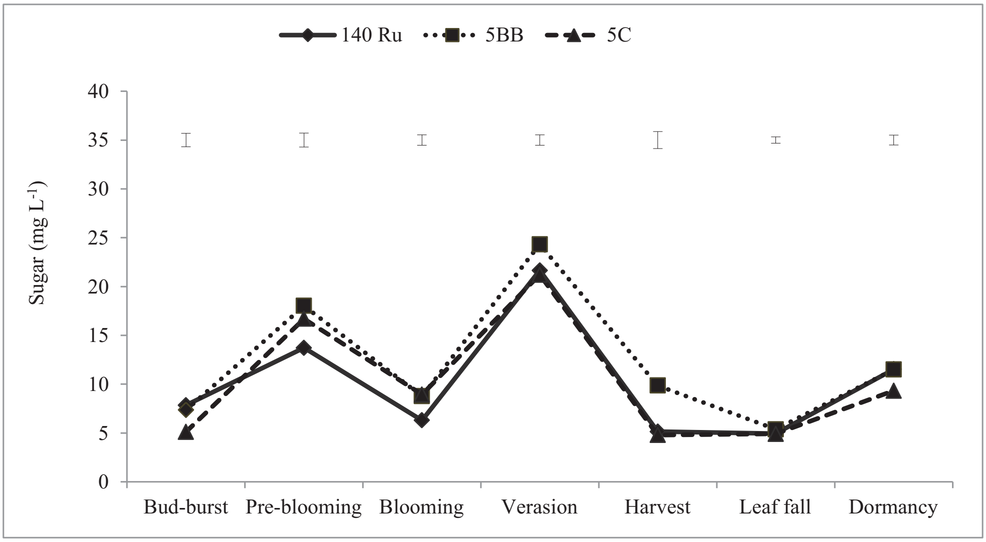


Figure 3. Seasonal changes of shoot sugar contents of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal p<0.01; rootstocks p<0.01)

In the study, the highest sugar content in annual shoots of grapes was identified as veraison stage. In general, sugar content of annual shoots at the end of growing season was higher in grapevines grafted on 140Ru rootstock than the other rootstocks. While sugar content of annual shoots of the grapevines grafted on 140Ru and 5BB rootstocks were almost at similar levels in budburst and dormancy periods, the sugar content of annual shoots of the grapevines grafted on 5C rootstock was lower in dormancy period than in previous period of budburst.

This situation may have stemmed from the earlier sugar conversion into starch in grapevines grafted on the 5C (Figure 3). Previous researchers indicated the reasons for such decreases as the high rate consumption of assimilation products during the blooming period (Dokoozlian, 2000; Lebon et al., 2008; Candolfi and Koblet, 1990) and the use of some of the sugar in sugar accumulation of the fruit and the conversion of sugars into starch for storage and usage in the development of new shoots in the subsequent spring (Winkler et al., 1974). A slight increase was determined in the sugar content of the shoots during the dormancy period. Such an increase may have resulted from the conversion of stored starch into sugar again for cold resistance in the dormancy period. Similar findings on conversion of stored starch in to against cold damage into sugar were also reported by previous researchers (Winkler and Williams, 1945; Hamman et al., 1996). Smith et al. (2009) reported that carbohydrate contents in entire tissues of grapevines decreased until blooming period and increased again between veraison and harvest periods.

Significant differences were also obtained in terms of starch content of annual shoots between rootstocks and phenological stages (p<0.01). In budburst stage, the highest starch content of shoots was determined in grapevines grafted on 140Ru rootstock (Figure 4).

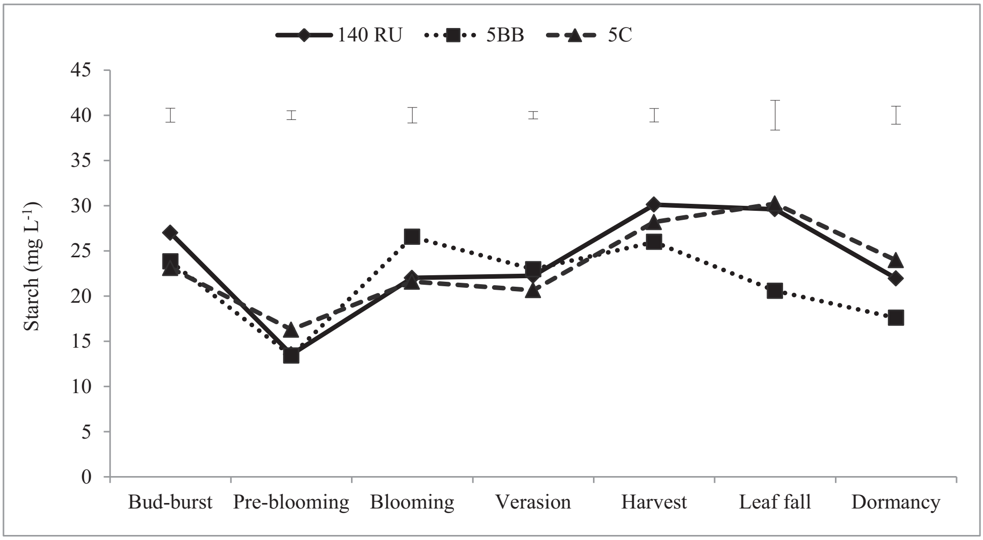


Figure 4. Seasonal changes of shoot starch contents of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

The starch contents of shoots were calculated as 26.99 mg L^{-1} on 140Ru, 23.81 mg L^{-1} on 5BB and 23.10 mg L^{-1} on 5C grafted grapevines in the bud burst stage.

The starch contents of new developed shoots were quite less on all rootstocks in pre-blooming period. During this period, newly developed leaves were still in consumer position and thus had relatively low assimilation capacity (Zamski and Schaffer, 1996).

The amount of starch continued to increase from pre-blooming to harvest period. In blooming period, the grapevines grafted on 5BB rootstock had the highest shoot starch content (Figure 4).

During the dormancy period, starch content of annual shoots was higher in grapevines grafted on 5C rootstock than in grapevines grafted on 5BB and 140 Ru rootstocks (Figure 4). Scholefield et al. (1978) indicated that sugar synthesized in vine leaves was converted into sugar following the harvest and stored in roots and stems to be used in shoot development of the subsequent spring.

Starch is the most significant carbohydrate providing winter resistance of xylem, especially of the roots of grapevines (Weyand and Schultz, 2006). Starch and soluble sugars are two main stored carbohydrates in grapevines (Mc Artney, 1998).

Entire leaves over the shoots are photosynthetic storage sources (Hunter and Visser, 1998). Therefore, active post-harvest leaves play significant roles in grapevines for sufficient storage of carbohydrates in roots to be used in the shoot development and flowering of the subsequent spring (Loescher et al., 1990).

Carbohydrates stored in vine canes are the indicators of grapevine health and vigor of the previous season (Balasubrahmanyam et al., 1978). Significant differences were observed in the total carbohydrate contents of annual shoots between rootstocks and phenological periods ($p<0.01$).

The amount of carbohydrate in bud burst period was higher in grapevines grafted on 140Ru than in grapevines grafted on 5BB and 5C rootstocks (Figure 5).

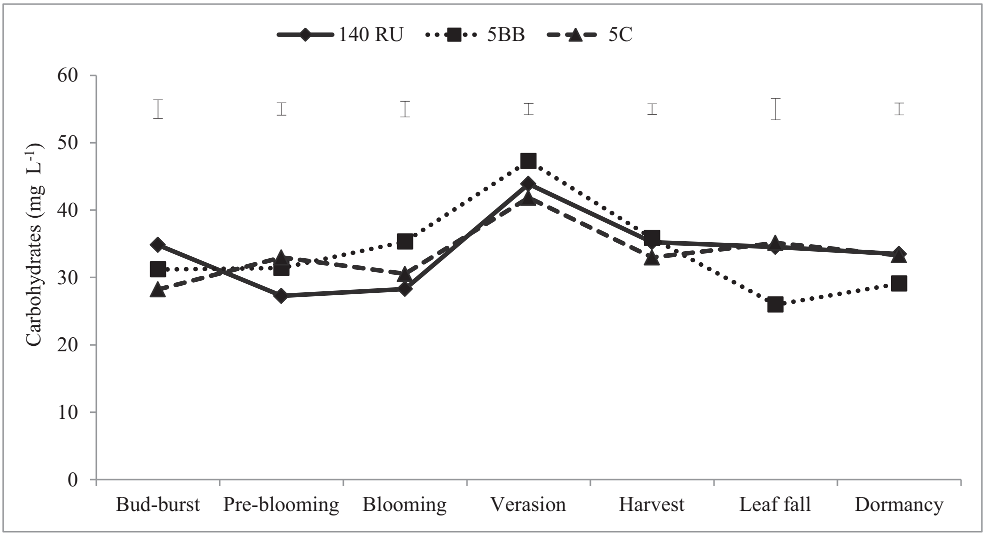


Figure 5. Seasonal changes of shoot total carbohydrate contents of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

In bud burst stage, carbohydrate contents were calculated as 34.84 mg L^{-1} on 140Ru, 31.18 mg L^{-1} on 5BB and 28.22 mg L^{-1} on 5C (Figure 5).

Total carbohydrate content of annual shoots in pre-blooming stage was higher in grapevines grafted on 140 Ru and 5BB rootstocks. However, carbohydrate content of annual shoots between blooming and harvest stages was higher in grapevines grafted on 5BB rootstock than the other grapevines (Figure 5). The highest mean carbohydrate content (41.56 mg L^{-1}) was calculated in grapevines grafted on 5BB rootstock at veraison stage.

Following the veraison period, decreases were observed in carbohydrate contents of annual shoots on all rootstocks. Shoot carbohydrate contents from the budburst to end of growth period were higher in grapevines grafted on 140Ru and 5BB rootstocks than grafted on 5C.

The carbohydrate contents of shoots increased and reached maximum level at the veraison stage. Thus, various researchers also reported increasing carbohydrate contents parallel to the recess in the shoot growth and increase in leaf area (Loescher et al., 1990; Kozlowski, 1992; Lakso et al., 2007). After veraison period, decreases were observed in shoot carbohydrate contents. Since new developed shoots exhibit rapid growth throughout pre-blooming period, carbohydrate accumulations of shoots were quite low. During fast growth period, grapevines use their own reserves and photosynthesis products are used for such a rapid growth and development.

During this period, it is understood that grapevine uses its own reserves as well as produced assimilates for rapid growth and development. Since carbohydrates are used for new shoot development between dormancy and blooming periods, decrease is observed in these periods. In this regard, Smith et al. (2009) stated that an increase in carbohydrates was expected until the end of the growing season. The carbohydrate level of annual shoots obtained in the dormancy period was close to the values in the bud burst period (Figure 5).

Storage carbohydrates are very important for the best shoots growth, flowering and fruit set. Also it is important to support of the storage reserves of grapevines to be used in a subsequent period as well as the formation of flower bud initiation. It was shown that accumulation of carbohydrates in shoots continuing after flowering to harvest. Carbohydrate transport and storage are related to photosynthesis capacity of grapevine canopy (Smith et al., 2009), stored carbohydrates decrease until fruit set (Wolstenholme and Whiley, 1997). Several researchers indicated varying annual carbohydrate contents of grapevines (Kliewer and Nassar, 1966; Winkler and Williams 1945).

Changes of leaf chlorophyll contents and growth characteristics

The changes in total leaf chlorophyll contents of grapes grafted on 140 Ru, 5BB and 5C rootstocks in blooming, veraison and harvest periods are presented in Figure 6.

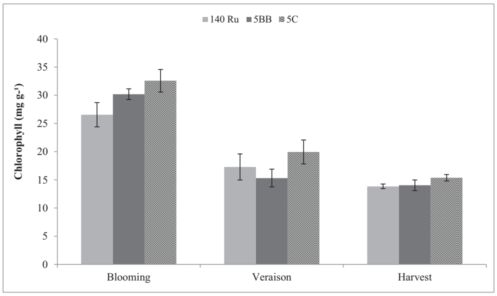


Figure 6. Seasonal changes of total chlorophyll contents of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.05$)

Significant differences were obtained in total chlorophyll contents in phenological periods ($p<0.01$) and rootstocks ($p<0.05$). The highest total chlorophyll content of grapevine leaves was calculated in blooming period. Besides, total chlorophyll content of grapevines grafted on 5C rootstock was higher than the other rootstocks in all three periods. In blooming period, leaf chlorophyll content was measured as 32.57 µg ml^{-1} for 5C, 30.19 µg ml^{-1} for 5BB and 26.58 µg ml^{-1} for 140 Ru. The total chlorophyll levels of the leaves decreased with the increase in leaf ages (Figure 6). Decreases were observed in the chlorophyll contents from veraison to harvest periods between all rootstocks. The lowest chlorophyll contents were determined in the harvest period in the all three rootstocks. The chlorophyll levels were relatively close to each other in the harvest period (Figure 6). In the study, the highest chlorophyll contents of leaves were determined in blooming stage. Poni et al. (1994) stated that leaf chlorophyll content was related to leaf age. Thus photosynthetic capacity of leaves was decreasing with increasing of leaf age.

Significant differences were obtained in terms of the mean and total leaf areas between rootstocks and phenological stages ($p<0.01$). Rapid increases were determined in mean and total leaf areas of grapevines grafted on all rootstocks until veraison period (Figures 7 and 8).

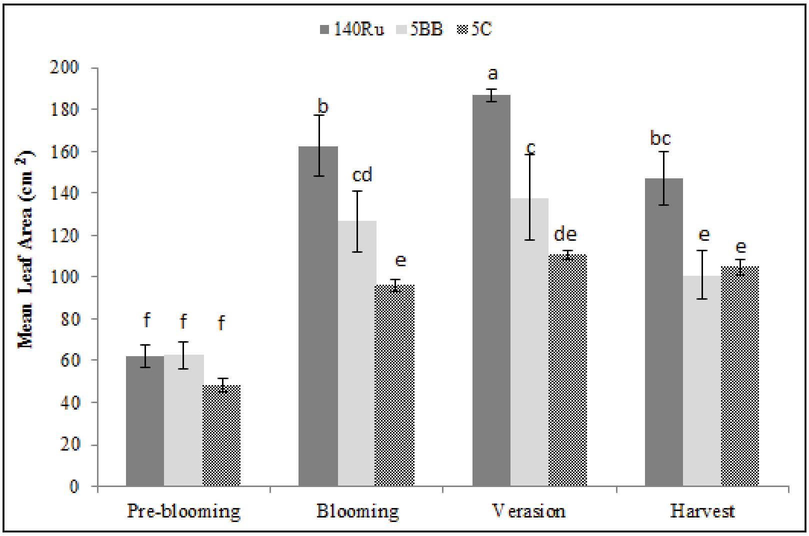


Figure 7. Seasonal changes of shoot mean leaf area (cm^2) of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

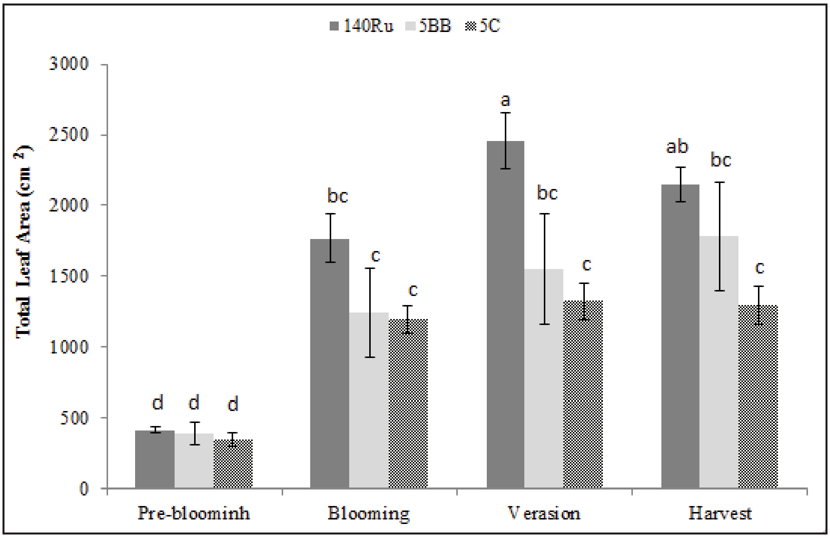


Figure 8. Seasonal changes of total shoot leaf area (cm²) of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

The highest total and mean leaf areas were observed in grapevines grafted on 140Ru rootstock. In veraison period, the highest mean leaf area was calculated as 187.0 cm^2 for 140Ru, 137.8 cm^2 for 5BB and 110.7 cm^2 for 5C. The total leaf areas in veraison period were measured as 2457.7 cm^2 for 140Ru, 1553.5 cm^2 for 5BB and 1326.0 cm^2 for 5C (Figure 8). In the study, the mean leaf areas of grapevines in blooming period reached to more than half of the maximum size they reached in veraison period. Maximum mean leaf area was calculated in veraison stage. The coincidence of increasing carbohydrate contents to the same period indicate the full photosynthetic capacity of the grapevine leaves.

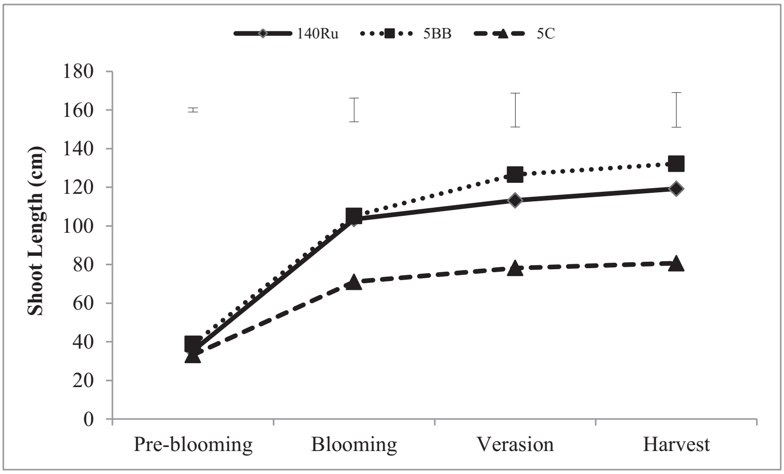


Figure 9. Seasonal changes of shoot length (cm) of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

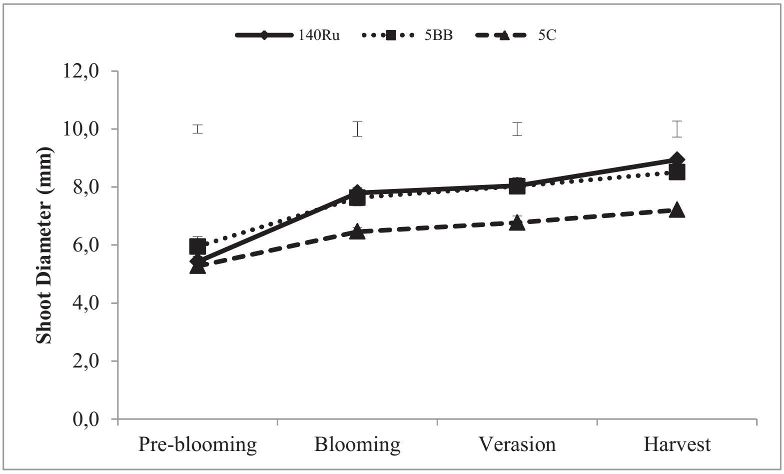


Figure 10. Seasonal changes of shoot diameter (mm) of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

A rapid increase was observed in shoot diameter and shoot length of grapevines grafted on all rootstocks until blooming period and that an increase slowed down after that period. After blooming, it was slowed down the growth of the shoot diameter and shoot length (Figures 9 and 10). Previous researchers also indicated that grapevines needed a significant amount of energy from the bud burst in spring to the veraison period (Hale and Weaver, 1962; Koblet, 1977) and new shoot development was dependent on self-reserves of the grapevines until a couple leaves reached at least half-size of the maximum leaf size (Hale and Weaver, 1962; Koblet, 1969; Winkler et al., 1974). Earlier studies revealed that photosynthetic products started to be transported to other organs when the grapevine leaves reached 30-50% of the maximum size (Koblet, 1977; Yang and Hori, 1980) or 50-75% of the maximum size (Koblet, 1969).

In the research, shoot diameter and shoot lengths showed significant differences in terms of between rootstocks and phenological stages ($p<0.01$). The changes in the shoot diameter and shoot lengths of grapes grafted on different rootstocks between pre-blooming and harvest periods are presented in Figure 9 and 10. At the end of the growth period, the greatest shoot length (132.2 cm) was determined in grapevines grafted on 5BB rootstock and the lowest value (80.6 cm) was seen in grapevines grafted on 5C rootstock (Figure 9). With regards to shoot diameters at the end of the period, the values were respectively observed as 8.9 and 8.5 cm in grapevines grafted on 140 Ru and 5BB rootstocks and as 7.2 cm in grapevines grafted on 5C rootstock (Figure 10). Current results revealed that 5C rootstock exhibited slower grow than 140Ru and 5BB rootstocks.

Yield and cluster characteristics

In the study, yield and some cluster characteristics are evaluated in Table 2. Total soluble solids content (TSSC-^{o}Brix), cluster weight and yield per vine showed significant differences as compared to rootstocks. While higher soluble solids were found on 5C grafted grapevines, mean cluster weight, berry weight and yield per vine was at on 5BB grafted grapevines (Table 2). Soluble solids content was calculated as between 19.1 and 20.6 ^{o}Brix. The highest TSSC was obtained 20.6 ^{o}Brix on 5C grafted grapevines. Cluster weight changed between 161.5 and 218.0 g. The heavier clusters and berries were produced on 5BB grafted grapevines. Total yields per vine were calculated between 8.3 and 12.3 kg according to rootstocks. The highest total yield per vine was obtained as 13.5 kg on 5BB grafted grapevines. Although 5C grafted grapevines were more successful in terms of soluble solid content, the heavier fruit clusters, berries and total yield per vine were produced on 5BB grafted grapevines (Table 2).

Table 2. The effect of rootstocks on yield and cluster characteristics of *Vitis labrusca* L. grape

Rootstocks	TSSC (°Brix)	Titrable Acidity (g/L)	Cluster Weight (g)	Berry Weight (g)	Yield (kg/vine)
140 Ru	19.1	8.7	202.6	3.3	12.3
5BB	19.6	9.2	218.0	3.6	13.5
5C	20.6	9.5	161.5	3.2	8.3
SEM	0.196	0.210	4.419	0.101	3.881
Rootstocks	**	ns	**	ns	**

(SEM; Standart Error of Means, ** p<0.01; ns: non-significant)

CONCLUSIONS

This study showed that stored carbohydrates consume for shoot growth, blooming and fruit set from blooming to veraison periods. The use of storage carbohydrates have been observed extensively until flowering period. Since the storage carbohydrates are very important for the shoots growth, flowering and fruit set, the cultural practices especially summer pruning which will be made before veraison stage are important for the accumulation of carbohydrates. In the study, 5BB rootstock was found to be suitable for shoot carbohydrate accumulation and growth characteristics of *Vitis labrusca* L. grape.

REFERENCES

Balasubrahmanyam V.R., Eifert J., Diofasi L., 1978.Nutrient reserves in grapevine canes as influenced by cropping levels. Vitis, 17(1): 23–29.

Bates T.R., Dunst R.M., Joy P., 2002. Seasonal dry matter, starch, and nutrient distribution in 'Concord' grapevine roots. HortScience, 37(2): 313–316.Boselli M., Fregoni M., Vercesi A., Volpe B., 1992.

Variation in mineral composition and effects on the growth and yield of Chardonnay grapes on various rootstocks. Agric. Ricerca 14: 138–139.

Candolfi-Vasconcelos M.C., Koblet W. 1990. Yield, fruit quality, bud fertility and starch reserves of the wood as a function of leaf removal in *Vitis vinifera*- Evidence of compensation and stress recovering. Vitis, 29(4): 199-221.

Caspari H.W., Lang A., Alspach P., 1998. Effects of girdling and leaf removal on fruit set and vegetative growth in grape. Am. J. Enol. Vitic., 49(4): 359-366.

Coombe B.G., 1995. Growth stages of the grapevine: adoption of a system for identifying grapevine growth stages. Aust. J. Grape and Wine Res., 1(2):104-110.

Dokoozlian N.K., 2000. Grape berry growth and development. In: Christenson, L.P. (ed.), Raisin production manual, pp. 30-37. University of California, Agricultural and Natural Resources Publication 3393, Oakland, CA.

Elsner E.A.. Jubb G.L., 1988. Leaf area estimation of Concord grape leaves from simple linear measurements. Am. J. Enol. Vitic., 39(1):95-97.

Ferroni G., Scalabrelli G., 1995. Effect of rootstock on vegetative activity and yield in grapevine. Acta Horticulturae 388: 37–42.

Göktürk B.N., Hallac F., Çetin S., Babalık Z., 2005. Investigation of Seasonal Carbohydrate Changes in Grapevine Cuttings. Turkey VI. Viticultural Symposium, Vol:2, p. 383-389, Tekirdağ (in Turkish).

Hale C.R., Weaver R.V., 1962. The effect of developmental stage on direction of translocation of photosynthate in *Vitis vinifera*. Hilgardia, 33: 89-13.

Hamman R.A., Dami I.E., Walsh T.M., Stushnoff C., 1996. Seasonal carbohydrate changes and cold hardiness of Chardonnay and Riesling grapevines. Am. J. Enol. Vitic., 47(1): 31-36.

Holzapfel B.P., Smith J.P., 2012. Developmental stage and climatic factors impact more on carbohydrate reserve dynamics of Shiraz than cultural practice. Am. J. Enol. Vitic., 63(3): 333–342.

Hunter J.J.. Visser J.H., 1988. Distribution of 14C-photosynthetate in the shoot of Vitis vinifera L. cv. Cabernet Sauvignon. II. The effect of partial defoliation. S. Afr. J. Enol. Vitic., 9(1): 10-15.

Jogaiah S., Oulkar D.P., Banerjee K., Sharma J., Patil A.G., Maske S.R., Somkuwar R.G., 2013 Biochemically Induced Variations During Some Phenological Stages in Thompson Seedless Grapevines Grafted on Different Rootstocks. S. Afr. J. Enol. Vitic. 34(1): 36-45.4: 36-45.

Kliewer W.M., Nassar A.R., 1966. Changes in concentration of organic acids, sugars, and amino acids in grape leaves. Am. J. Enol. Vitic., 17(1):48-57.

Koblet W., 1969. Wanderung von Assimilaten in Rebtrieben und Enfluss der Blattflache auf Ertrag und Qualitat der Trauben (Habilitationsschrift). Wein-Wiss., 24:277-319.

Koblet W., 1977. Translocation of photosynthate in grapevines. In : Proc. Int. Symp. on the Quality of the Vintage. 14-21 Feb. Cape Town, pp. 45-51.

Kozlowski T.T., 1992. Carbohydrate sources and sinks in woody plants. The Botanical Rev., 58(2): 107-222.

Köse B., Karabulut B., Ceylan K. 2014. Effect of Rootstock on Grafted Grapevine Quality. Europ. J.Hort.Sci. 79(4):197–202.

Lakso A.N., Intrigliolo D., Eissenstat D.M., 2007. Modeling concord grapes with "VitiSim", a simplified carbon balance model: understanding pruning effects. In VIII International Symposium on Modelling in Fruit Research and Orchard Management, 803: p.243-250.

Lebon G., Wojnarowiez G., Holzapfel B., Fontaine F., Vaillant-Gaveau N., Clement C., 2008. Sugars and flowering in the grapevine (*Vitis vinifera* L.). J. Exp. Bot., 59(10): 2565–2578.

Lichtenthaler H.K., Wellburn A.R., 1983. Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvent. Biochem. Soc. Trans., 603(11): 591-592.

Loescher W.H., Mc Camant T., Keller J.D., 1990. Carbohydrate reserves, translocation, and storage in woody plant roots. HortScience 25(3): 274-281.

MC Artney S.J., 1998. Remobilization of stored carbohydrates and nitrogen and their importance for initial development of grapevine shoots. Thesis. Ph.D., pp. 340. The Ohio State University, U.S.A

Nikolaou N., Angelopoulos K., Karagiannidis N., 2003. Effects of drought stress on mycorrhizal and non-mycorrhizal Cabernet Sauvignon grapevine, grafted onto various rootstocks. Exp. Agric., 39(3): 241-252.

Poni S., Intrieri C., Silvestroni O., 1994. Interactions of leaf age, fruiting, and exogenous cytokinins in Sangiovese grapevines under non-irrigated conditions. II. Chlorophyll and nitrogen content. Am. J. Enol. Vitic., 45(3):278-284

Rafaat El-Gendy S.S., 2013. Evaluation of Flame Seedless Grapevines Grafted on Some Rootstocks. J. Hortic.Sci., Ornam. Plants, 5(1):1-11.

Scholefield P.B., Neales T.P., May P., 1978. Carbon balance of the sultana vine (*Vitis vinifera* L.) and the effects of autumn defoliation by harvest pruning. Austral. J. Plant Phys. 5(5):561–570.

Scott T.A., Melvin E.H., 1953. Determination of dextran with anthrone. Analyt. Chemist., 25(11):1656-1661.

Smith J.P., 2004. Investigations into the mechanisms underlying grapevine rootstock effects on scion growth and yield. PhD Thesis, Charles Sturt University, Wagga Wagga, Australia, pp.196.

Smith J., Rogiers S., Quirk L., Holzapfel B., 2009. Management of carbohydrate reserve accumulation as a tool for regulating vine productivity and fruit quality. Australian Government, Grape and Wine Development Corporation Final Report.

Somkuwar R.G., Taware P.B., Bondage D.D., Navale S., 2012. Root length, root mass, and distribution of dry matter in different parts of Thompson Seedless grapevine grafted on different rootstocks in heavy soil of Maharashtra. Turk. J. Agric. Forest., 36(5):543-552.

Van der Zijpp S., Creagh T., 2011. Grape Physiology. Course Notes, Eastern Institute of Technology National Certificate in Horticulture, Faculty of Science and Technology, EIT Hawke's Bay, pp. 92.

Verma S.K., Singh S.K., Krishna H., 2010. The effect of certain rootstocks on the grape cultivar 'Pusa Urvashi' (*Vitis vinifera* L.). Int. J. Fruit Sci., 10(1):16–28. spacja

Weaver R.J., 1976. Grape Growing. John Wiley and Sons, New York, USA, p.371

Weyand K.M., Schultz H.R., 2006. Long-term dynamics of nitrogen and carbohydrate reserves in woody parts of minimally and severely pruned Riesling vines in a cool climate. Am. J. Enol. Vitic., 57(2):172–182.

Winkler A.J., Williams W.O., 1945. Starch and sugars of Vitis vinifera. Plant Physiol., 20(3):412-432.

Winkler A.J., Cook J.A., Kliewer W.M., Lider L.A., 1974. General viticulture. Univ. of Calif. Press, Berkeley, p. 710.

Wolstenholme B.N., Whiley A.W., 1997. What do carbohydrate reserves tell us about avocado orchard management? South African Avocado Growers' Association Yearbook 20: 63-67.

Yang Y.S., Hori Y., 1980. Studies on translocation of accumulated assimilates in Delaware grapevines III. Early growth of new shoots as dependent on accumulated and current year assimilates. Tohoku J. Agric. Res., 31(2): 120-129.

Zamski E., Schaffer A.A., 1996. Photoassimilate Distribution Plants and Crops Source-Sink Relationships. Marcel Dekker. New York-Basel-Honkong, 905 pp.

Zufferey V., Murisier F., Vivin P., Belcher S., Lorenzini F., Spring J.L., Viret O., 2012. Carbohydrate reserves in grapevine (*Vitis vinifera* L. 'Chasselas') influence of leaf to fruit ratio. Vitis, 51(3):103–110.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF TEMPERATURE AND HUMIDITY IN BLOOMING PHENOPHASE CONCERNING ON FRUIT SET IN SOME TABLE GRAPES (*VITIS VINIFERA* L.)

Marinela Vicuţa STROE, Toniţa Valentina DUNUŢĂ,
Daniel Nicolae COJANU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Department of Bioengineering of Horticulture-Viticulture Systems,
59 Marasti Blvd., District 1, 011464, Bucharest, Romania,
Phone: +40 21 318 36 36/216

Corresponding author email: marinelastroe@yahoo.com

Abstract

The aim of this study was to determine the influence of temperature and humidity on blooming phenophase and also on the percentage of fruit set of three table grapes varieties such as Muscat Hamburg cv., Afuz Ali and Victoria cv. (Vitis vinifera L*). For assessing the optimal timing analysis it was used a grading system called BBCH which is a universal scale for describing monocots and dicots numbered from 00-97, a special focus was made on the principal growth stage blooming and includes stages 61 and 67 and growth stage of fruit development stages 71 and 77. Results shows that there was a direct correlation between the average values of two analyzed factors and percent of fruit set, and also the size of fruits. Average temperatures below + 20°C during blooming and a relative humidity of 65% has determined a good percentage binding at Muscat Hamburg (63%), followed by Afuz Ali variety (80%) and Victoria variety (86%). Surprisingly, even though the last two variety have formed more berries during the process of fecundation, proportion of very small berries (2-4 mm) and small berries(6-7mm) was high situated between 51.07% and 55.24%.*

Key words*: blooming, grows, phenology, table grape, variety.*

INTRODUCTION

In the last decade, world production and consumption of fresh table grapes registered a noticeable increase Moreover, the consumer market, both globally and nationally, had a significantly increased every year, drawing attention to ensure competitive products in terms of sensory characteristics but also a high (OIV, 2013, Rolle et.al., 2015).

To achieve a competitive production, which means a good quality and high yield, a number of factors are related mainly environmental (Shinomiya et al., 2015) and climate change (Jones, 2005), and also genetic varieties and applied technology Among the environmental factors involved in plant development cycles, phenological temperature is considered to be a key factor that can profoundly alter the timing entire spectrum phenology for different plant species but also within the same species in different varieties (Parker et al., 2013). Phenology of varieties is changeable not only in terms of temperature impact but also by a complex interaction between temperature, humidity, precipitation, hour of insolation, registered at the beginning of each phenophases (Chuine et. Al., 2003, Cleland et al., 2007). From the factors mentioned above, blooming phenophases and fruit set are the most influenced. Blooming phenophase - after Baggiolini is stage I, and stage 65 after scoring system BBCH, (www.diprove.unimi.it/GRAPENET/index.php) or stage 23 after scoring system made by Eichorn and H. Lorenz, (1977), (Pierot and Rochard, 2013), starts about 55- 65 days after bud stage, when flowers and inflorescences are fully developed (Stroe, 2014). Blooming period can last, depending on variety and climatic conditions, between 7-16 days. In northern areas this phenophase can lasts 16 days and it starts when average temperature is approximate 17,5°C, in the south vineyards can lasts only 12 days, and in the centre of the country where the thermal regime is characterized by a warm temperate climate (Savu and Stroe, 2005) phenological phase lasts 7-8 days. In practice, it was observed that in the first 2-3 days after onset of blooming there was a percent of 20-30% open flowers, in the coming 3-4 days, 60-70% bloom and only a small percentage of flower bloom at the end phenophase, but in the inflorescence, the first flowers whom are opening are the one in the middle of it, followed by those from the base and, in the end, the top inflorescence (Mustea, 2004). Fruit set is a process of pollination and fertilization, a phenomenon influenced by biological, physiological, climate and technological factors.

Among the biological factors the most important is type of flowers, morphologic and functional, and also ability of pollen germination. Depending on the unfavorable conditions and fecundation during blooming the percentage of fruit set will be different, and berries will have a different evolution: very small berries is the lack of fecundation of flowers, berries remain small as a grain of millet after reaching 2-4 mm in diameter they do not increase, acquires a yellow-green base and forms a isolator layer, and therefore it occurs shaking berries (Dobrei et. al., 2005).

Millerandage is a common thing among grape varieties that require foreign pollen, and in this case, the berries stop growing the size of a peppercorn because of the lack of hormonal substances. Later, they mature earlier, and also can accumulate higher amounts of sugars and bring a good influence in production of quality wine grape varieties, but for quality of grape table varieties is a disadvantage. Even so, varieties studied in this paper are adapted to temperate continental climate in Romania they have a different response reaction in terms of the percentage of fruit set (Table 1).

Basically, adverse climatic conditions which means low temperatures (below 20°C), high humidity 55-65%, heavy rainfall - manifested in this phenophase can trigger a massive shaking of flowers, resulting in the formation of rare berry cluster, which occurs most often the millerandage phenomenon.

Studies have shown that percentage of fruit set is an average of 36% to Rhine Riesling, Gewurztraminer from 35%, 66% at Italian Riesling, 37% to Chasselas and 65% to Oporto (Irimia, 2012).

This phenomenon must not be mistaken with pollination and fertilization disfunctions, due to the fact that, in the first case, it represents a sugar redistribution disorder, with different effects depending on the period when it is initiated (Bernaz, 2003).

Under this circumstances, May occur other physiological diseases caused by either lack or excess of nutrients during some phases of the anual vegetative cycle.

Based on this consideration, the paper aim was to analyzed the influence and effect of temperature and humidity on fruit set development in the climatic conditions of the year 2016.

Results of this experience were analyzed under the quality parametres of a high yield/commodity production.

MATERIALS AND METHODS

Studied varieties and growth conditions

In this study were analyzed three varieties of table grapes: Muscat Hamburg cv., Afuz Ali cv., from the world's assortment and Victoria cv., a romanian variety obtained in 1978 by Victoria Lepădatu and Gh. Condei. The main data about these varieties can be found in Vitis International Variety Catalogue (www.vivc.de) and The European Vitis Database. In vineyard, all these three varieties have proven over the years, requiring a specific temperature and humidity during blooming phenophase. Table grape 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 30 buds/vine. During experiment observations and measurements were currently made for determining the elements of fertility and productivity, with a special focus was made on carpometry indices that define the productive potential: % fertile shoots, absolute fertility coefficient, relative fertility coefficient, absolute productivity index (g/shoot), relative productivity index (g/shoot), number of grape/vine, average weight of a grape (g), yield (kg/vine).

Short presentation of Muscat Hamburg variety. Is a variety with medium vigor growth holding up relatively well at lower temperature during winter and has a good fertility manifested by 70% fertile shoots. In normal years, the flowers have the capacity to fruit set up to 50% because the capacity of pollen germination is small, and that is why indicated to cultivate in vineyard with alongside varieties like Chasselas doré and Afuz Ali (Stroe, 2012).

Short presentation of Afuz Ali variety

Hardy variety, but sensitive to low winter temperatures (-16 °C, - 18 °C) with a long growing season 180-210 days, that is why fails to mature wood well. It has a great sensitivity to frost and this leads to poor resistance to diseases like bacterial cancer cancer and anthracnose.

Short presentation of Victoria variety

Variety with a medium to high vigor and has a good fertility, manifested by a percentage of 63-73% fertile shoots. Variety is characterized by good resistance to frost (-20 °C) and drought, it behaves well towards spring frosts because of late budbreak. It has an average resistance to mildew and very poor resistance to powdery mildew (Stroe and Veliu, 2010).

Phenological and temperature data

In this study it was used a update version of BBCH, an universal scale used for describing monocots and dicots numbered from 00-97, with special focus on the principal growth stage 6: Flowering, stages 61 to 67 and principal growth stage 7: Development of fruits, 71 to 77 (Pierot and Rochard, 2013). Three observations were made on all inflorescence of 6 vine for each variety separately in different stages of development as shown in table 2.

Meteorological parameters were analyzed in the period 20 May-20 June 2016 using daily averages of 6 points hourly results of the day (5:00, 08:00 11:00 14:00 17:00 20:00 23:00) it was recorded by weather station Bucharest - Baneasa, Romania. The calculation was conducted on a higher timeframe, to ensure better accuracy of results, even so phenophase of blooming at studied varieties lasted 12 days (20 - 31 May 2016).

RESULTS AND DISCUSSIONS

Although well adapted to the temperate continental climate from Romania, the varieties Muscat Hamburg cv., Afuz Ali and Victoria cv. responded differently in vineyard in what concerns the percent of fruit set, the pergentage of formed flowers shaken, and also the millerandage phenomen, which in particular years can put their marks on the obtained crops. Climatic particularities of the wine year 2016 resulted in earliness in flowering terms Cleland (2007), noticing that the varieties analysed bloomed in the third decade of May (Figure 1) within a period of 12 days (20 - 31 May 2016).

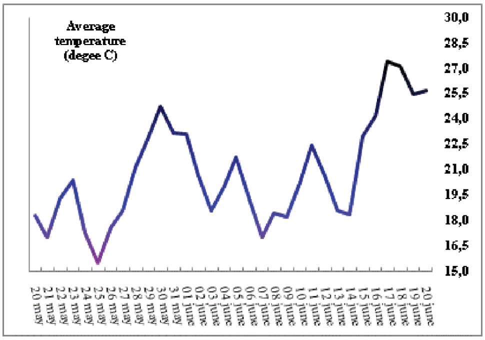


Figure 1. Evolution of the average temperature (°C) for period 20 May - 20 June 2016

In this amount of time, the average daily temperatures recorded presented in figure 1 were of 19.69°C with an inferior limit of 15.5°C recorded in 25th of May and a maximum of de 20.4°C in 23th May, bassically, when the blooming phenophase was at its best, beeing well known that in the first days from the flowering 20-30% of the total flowers, in the following days are flowering 60-70% and only a small percentage open at the end of the phenophase Mustea (2004).

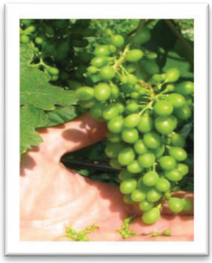
Regarding values of relative humidity (%) figure 2 gives an indication of their impact on analyzed varieties, because in the same period, the average was 70.65% with a peak of 83.12% in May 25th, values far exceeding the normal limit of blooming knowing that optimal conditions is in range 55-65%.

Following the recordings in figure 3, we notice that the percent of the fruit set is different from a variety to another, the Muscat Hamburg variety records the smallest percent (63%), followed by the Afuz ali variety (80%) and then Victoria variety (86%).

Table 1. The genetic origin of studied varieties

Prime name	**Muscat de Hamburg**	**Afuz Ali**	**Victoria**
Variety number VIVC	8226	122	13031
Country of origin of the variety	U. K.	Liban	România
Species	*Vitis vinifera* L.	*Vitis vinifera* L.	*Vitis vinifera* L.
Pedigree as given by breeder/bibliography	Trollinger x Muscat de Alexandria	-	-
Pedigree confirmed by markers	Schiava grossa x Muscat Alexandria	-	Cardinal x Afuz Ali
Prime name of pedigree parent 1	Schiava grossa	-	Cardinal
Prime name of pedigree parent 2	Muscat Alexandria	-	Afuz Ali
Year of crossing	1850	-	1964
Last update	18.01.2017	18.01.2017	18.01.2017

Table 2. Phenological study of vine during blooming and development of fruits

Principal growth stage 6: Flowering (BBCH MODIFIED PHENOLOGICAL SCALE FOR COST ACTION FA1003)		
6.1: Beginning of flowering: 10% of flowerhoods fallen		
6.7: 70% of flowerhoods fallen		
Principal growth stage 7: Development of fruits		
7.1: Fruit set: young fruits begin to swell, remains of flowers lost		
7.7: Berries beginning to touch (if bunch are tight)		
I.Observation (30.05.2016) 70% of flowerhoods fallen 	II. Observation (05.06.2016) Small-berry grape only formats 	III. Observation (01.07.2016) Berries beginning to touch 

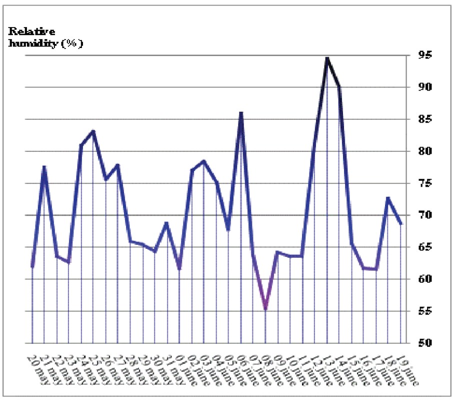


Figure 2. Evolution of the average relative humidity (%) for the period 20 May-20 June 2016

Analysing figure 4 which give details about size categories in which the formed berries belong, expressed as a percentage, it is observed that the Muscat Hamburg variety stands out with a great percentage of normal berries (65.9%), variety specific, followed by Victoria variety with 49.10% and then we find the Afuz Ali variety with a lower percent of 44.69%. It is surprisingly that even if the last varietes formed more berries in the process of fecundation, the proportion of berries remained very small (millet dimensions of 2-4 mm) and small (peppercorn dimensions 6-7 mm) was highly pronounced (55.24% and 51.07%).

All this results are affecting in a negative way the crops obtained (Table 3), literally, these shows a low productive potential, under the limit of these variety potential, which is given by the high percentage of very small and small berries remained.

A similar evolution was recorded in what concerns the average weight of a grape (220 g/grape - Muscat Hamburg cv., 270 g/grape - Afuz Ali cv. and 281g/grape Victoria cv.), determining a low yield, between 2.24-4.05 kg/vine, and the merchandise production in this case is being the lowest Stroe et al. (2016).

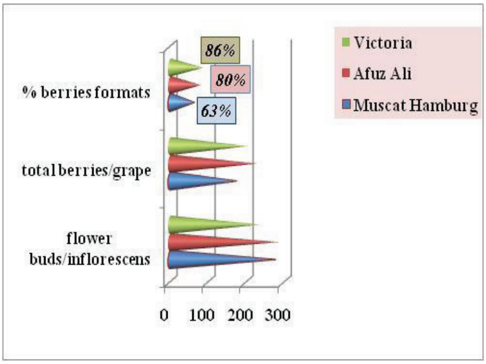


Figure 3. Evolution of the berries formats (%) for the period 20 May -20 June 2016

CONCLUSIONS

The average temperature and humidity values recorded in the blooming phenophase have left their mark on the three varieties studied, demonstrating through the obtained results that they are very pretentious and requires in this phenophase higher temperatures (over 20°C) and relative moist values of 65%.

Regarding the categories of the formed berries was recorded that Afuz Ali and Victoria varieties although formed much more berries, about half of them remained small (6-7 mm) and very small (2-4 mm).

Although Muscat Hamburg is known as a variety with millerandage problems in the given conditions of 2016, this was exceeded in this particular study of Afuz Ali and Victoria varieties, Victoria and Afuz Ali varieties are having in its pedigree Cardinal variety.

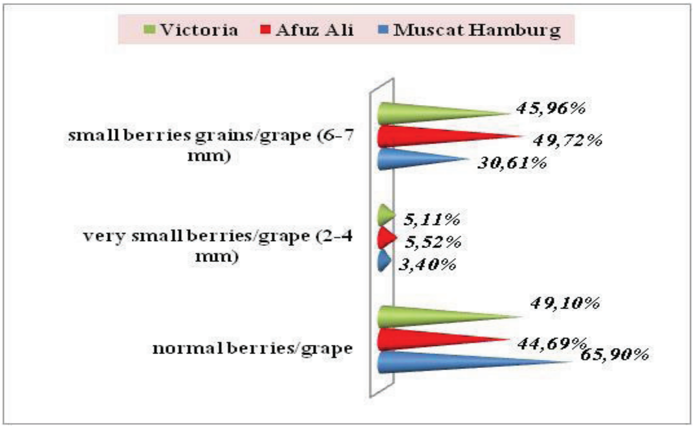


Figure 4. Evolution of the categories of grains formed of studied varieties

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

Varieties	% fertile shoots	Absolute fertility coefficient	Relative fertility coefficient	Absolute productivity index (g/shoot)	Relative productivity index (g/shoot)	No. of grapes/vine	Average weight of a grape (g)	Yield (kg / vine)
Muscat Hamburg	58.33	0.91	1.57	200.2	345.4	11	220	2.42
Afuz Ali	61.11	0.83	1.36	348.6	571.2	15	270	4.05
Victoria	50.00	0.80	1.33	240	399	8	281	2.24

For an improvement, as a tehnological intervention, it can be applied several actions intervening on the sugar redistribution system: pinching fertile shoots a few days before blooming, annular incision below the inflorescence, application of growth retardant treatments, to briefly stop vegetative growth and redirect nutrients to inflorescences, balanced fertilization with potassium, which favors the sugars migration and accumulation of reserves, and also reducing nitrogen fertilizer rates.

REFERENCES

Bernaz Gh., 2003. Refacerea viilor vătămate de accidente climatice și boli fiziologice-Gh. Editura M.A.S.T 2003, 26-27.

Chuine, I., Kramer, K., Hanninen, H., 2003. Plant development models. Phenology:An Integrative Environmental Sciences, vol. 39, 217–235.

Cleland, E.E., Chuine, I., Menzel, A., Mooney, H.A., Schwartz, M.D., 2007. Shifting plant phenology in response to global change. Trends Ecol. Evol. 22 (7), 357–365.

Dobrei Alin, Liliana Rotaru, Mihai Mustea, 2005. Cultura viţei de vie. Editura Solness, Timişoara, 62.

Irimia L. M., 2012. Biologia, ecologia şi fiziologia viţei-de-vie. Editura "Ion Ionescu de la Brad", Iaşi, 79, 87.

Isabelle Pierot, Joël Rochard, 2013. Adaptation aux changements climatiques - projet europeen Leonardo Da Vinci: E-viticlimate. The XXXVI-th World Congress of Vine and Wine June 2-7-th 2013, Bucharest. ISBN O.I.V. 979-10-91799-16-4.

Jones G. V., White M. A., Cooper O. R., Storchmann K., 2005. Climate change and global wine quality. Climatic Change 73, 319-343.

Luca Rolle, Fabrizio Torchio, Simone Giacosa, Susana Río Segade, 2015. Berry density and size as factors related to the hysicochemical characteristics of Muscat Hamburg table grapes (Vitis vinifera L.). Journal of Food Chemistry, 173 (2015), 105-113.

Mustea M., 2004. Viticultură - Bazele biologice, înființarea și întreținerea plantațiilor tinere de vii roditoare. Editura "Ion Ionescu de la Brad", Iaşi, 151-153, 157.

O.I.V., 2013. OIV vine and wine outlook 2008–2009. Paris: Organisation Internationale de la Vigne et du Vin.

Parker Amber et al., 2013 - Classification of varieties for their timing of flowering and veraison using a modelling approach: A case study for the grapevine species Vitis vinifera L. Agricultural and Forest Meteorology 180 (2013) 249 – 264.

Ryo Shinomiya et al., 2015. Impact of temperature and sunlight on the skin coloration of the 'Kyoho' table grape. Scientia Horticulturae 193 (2015) 77–83.

Savu Georgeta Mihaela, Stroe Marinela Vicuţa, 2005. Evaluarea condiţiilor ecoclimatice, cu ajutorul unor indicatori sintetici, în regiunea viticolă a Dealurilor Munteniei şi Olteniei. Simpozion ştiinţific anual, "Horticultura – ştiinţă, calitate, diversitate-armonie", U.S.A.M.V. „Ion Ionescu de la Brad", Iaşi, 27-28 mai, XLVIII Vol 1 (48), Seria Horticultură, CD-ROM I.S.S.N. 1454-7376, 303-308.

Stroe Marinela, 2014. Lucrări practice Ampelografie. Editura Invel-Multimedia, Ediţie revăzută şi adăugită, 2014, ISBN 978-973-1886-80-0, 7, 15.

Stroe Marinela Vicuţa, Raluca Veliu, 2010. The agrobiological and technological evaluation of some table grape variety with different maturation periods in vineyard Ostrov. Sesiunea Ştiinţifică anuală „Horticultură - Ştiinţă, Calitate, Diversitate şi Armonie", U.S.A.M.V. „Ion Ionescu de la Brad" Iaşi, Facultatea de Horticultura, Anul III, Vol 53, Seria Horticultură, CD ROM ISSN 2069-847X, 437- 443.

Stroe Marinela, 2012. Ampelografie. Editura Ceres, Bucureşti, ISBN 978-973-40-0943-5, (COD CNCSIS 236), 187, 193, 203.

Stroe Marinela, Bejan Carmen, Carmen Florentina Popescu, 2016 - Research regarding of the technological parameters of some table grape varieties in the experimental field from U.A.S.V.M. Bucharest - Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture, vol. 73, no 1 (2016), ISSN 1843-5254.

The European Vitis Database, Genetic resources of grapes, website, www.eu-vitis.de/index.php, accessed on February 2017.

Vitis International Variety Catalogue, Passport of variety Muscat Hamburg, Variety number VIVC 8226, passport of Afuz Ali, VIVC number 122, passport of Victoria cv, VIVC number 13031, website, http://www.vivc.de, accessed on ianuary 2017.

http://www.diprove.unimi.it/GRAPENET/index.php, Cost action FA1003: East-West collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding, PHENOTYPING TRIAL 2012, First circular 12th March 2012.

VEGETABLE GROWING

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE REACTION OF TOMATO GENOTYPES TO FUNGAL PATHOGENS UNDER CONTROLLED CONDITIONS

Nadejda MIHNEA, Galina LUPAŞCU, Irina ZAMORZAEVA

Academy of Sciences of Moldova, Institute of Genetics, Physiology and Plant Protection, 20 Pădurii Street, MD-2002, Chisinau, Republic of Moldova

Corresponding author email: mihneanadea@yahoo.com

Abstract

The paper presents results of the reaction of new tomato varieties and perspective lines created in the Institute of Genetics, Physiology and Plant Protection to the seeds treatment with FC Fusarium oxysporum, F. solani, F. redolens, Alternaria alternata based on the estimation of seeds germination and length of embryonic radicles and stemlets. It should be mentioned that the reaction of some varieties to same isolates was quite different. Thus, in the case of seeds germination the capability of some approved varieties after treating them with FC of fungi, the highest resistance was found for varieties 'Exclusiv', 'Desteptarea', 'Milenium'. Variety 'Mihaela' has demonstrated the highest complex resistance to FC of 4 used fungi in the case of the reaction of radicles and stemlets, the phenomenon was manifested as insignificant reduction or stimulation. Using bifactorial analysis of variance we found that the most contribution to the variation of embryonic radicle and stemlet length had the species of fungus, being 41.07 and 58.58%, respectively. It should be mentioned that the genotype plays an important role too, its factorial pondering consists of the 32.49 and 22.00%, respectively, for the length of radicles and stemlets, and the interaction tomato genotype x fungus species was recorded as 25.03 and 17.73%, respectively, for both characters. The significant pondering of the influence of fungus species (Fusarium spp., A. alternata) and the interaction tomato genotype x species of fungus on the source of variation of plant growth organs reveals the necessity of constant monitoring of the composition of pathogen species and their virulence.

Key words: *tomato, resistance, fungal pathogens, Fusarium spp., A. alternata.*

INTRODUCTION

The resistance of agricultural crops to biotic and abiotic stressful factors is one of the basic requirements to contemporary varieties, including varieties of tomato. The problem of complex and sustainable resistance is real for many crops, but especially for tomato because their fruits are extensively used in ordinary food or dietary management of children and elderly people that is why the application of chemical plant protection has to be limited. In the Republic of Belarus fungal diseases may cause losses of 40-60% of tomato crops, in some years even of 80% (Polixenova, 2009).

Treatment with fungicides is quite difficult to optimize or make more efficient because as the capacity of plant infection as well as the efficiency of preparations may be manifested only in favorable climatic conditions that cannot often be forecasted.

The diseases are easily transmitted from one plant to another, spreading extensively on large surface. Tomatoes are susceptible to 200 fungal, bacterial, viral diseases and nematodes. The most common diseases are root rot, *Fusarium* wilt, *Verticillium* wilt, blight, various bacterial and viral infections, for which some sources of resistance are identified (Foolad, 2007; Scott Gardner, 2007).

Toxins of fungi *Alternaria* spp. and *Fusarium* spp. are often involved in the pathogenesis, accumulating in tomato fruits, especially of susceptible varieties, and making them quite toxic for human (Yamagishi et al., 2006).

Performing agricultural techniques, biological control, resistant varieties, chemical treatments are considered as basic factors of effective measures for the control of tomato diseases. Lifetime of the resistant varieties which are usually recommended for production is often limited by the emergence of new races of different pathogens exceeding the resistance of genes of cultivated varieties (Amini, Sidovich, 2010).

Chemical control of diseases is usually effective, but it has some unintended consequences as the reduction of amount of many beneficial organisms, as well as toxic impacts on human health and environment (Manczinger, Antal, Kredics, 2002; Gavrilescu, Chisti, 2005).

From the point of economic view and ecological advantageous the use of varieties with long-term resistance for the control of tomato diseases produced by fungi is preferable.

The aim of our study was to test the level of resistance of new tomato varieties and lines to some fungal diseases (*Fusarium* spp., *A. alternata*) for the selection of forms with enhanced resistance.

MATERIALS AND METHODS

Six varieties and twelve perspective lines of tomato, created in the Institute of Genetics, Physiology and Plant Protection (IGPPP) and manifested the complex of valuable characters, were used as a material for research.

Seeds of tomato were treated with FC of the fungi for 18 hours. Seeds, which were contained in distilled water, were used as a control. The cultivation of seedlings was carried out in Petri dishes on the filter paper, moistened with distilled water, at room temperature 22-24ºC for 6 days. The important characters of growth and development of tomato at the early stages of ontogeny – germination, length of radicle and length of stemlet – were used as index-test of the reaction of plants.

In order to elucidate the features of the action of fungi *F. oxysporum, F. solani, F. redolens* and *A. alternata* on the ability of germination and growth of radicle and stemlet of tomato the analysis Box & Whisker Plot was made.

Cluster analyses were performed by creating dendrograms on the base of agglomerative-iterative algorithm using Ward method and the method *k*-means (Savary, 2010).

Bifactorial analysis of the variance ANOVA was applied to appreciate the role of the genotype, the species of fungus, and their interaction as a source of variation of quantitative traits.

RESULTS AND DISCUSSIONS

Analysis of the reaction of varieties and perspective lines of tomato to seeds treatment with the FC of fungi *F. oxysporum, F. solani, F. redolens* and *A. alternata* isolated from roots and leaves with symptoms of disease allowed to find that the reaction of plants to all four FC was different, specific for genotype, analyzed character, and species of fungus. Responses to infection were classified to the categories: inhibition, stimulation, lack of reaction (Table 1).

Table 1. The influence of culture filtrates of *Fusarium* spp. and *Alternaria alternata* on some characters of tomato growth and development

No.	Variant	Germination, %	% relative to control	Length of radicle, mm	% relative to control	Length of stemlet, mm	% relative to control
			'Tomis'				
1.	H_2O (control)	100.0	100.0	37.7±1.8	100.0	20.8±1.0	100.0
2.	FC *F. oxysporum*	88.3	88.3	32.6±2.4	86.5	15.6±1.2*	75.0
3.	FC *F. solani*	48.3	48.3	27.9±3.9*	74.0	26.8±3.1	128.8
4.	FC *F. redolens*	81.7	81.7	29.6±3.1*	78.5	19.1±2.5	91.8
5.	FC *A. alternata*	78.3	78.3	16.2±1.6*	43.0	12.2±1.2*	58.6
			'Exclusiv'				
1.	H_2O (control)	98.3	100.0	38.3±2.3	100.0	25.4±1.1	100.0
2.	FC *F. oxysporum*	91.5	93.1	15.5±1.3*	40.5	10.2±0.8*	40.2
3.	FC *F. solani*	71.2	72.4	13.6±1.6*	35.5	14.3±2.0*	56.3
4.	FC *F. redolens*	89.8	91.3	25.6±3.1*	66.8	15.8±1.9*	62.2
5.	FC *A. alternata*	100	101.7	31.3±2.6	81.7	18.6±1.5*	73.2
			'Mihaela'				
1.	H_2O (control)	100.0	100.0	37.4±2.0	100.0	19.5±1.2	100.0
2.	FC *F. oxysporum*	90.0	90.0	35.8±3.1	95.7	18.5±1.6	94.9
3.	FC *F. solani*	55.0	55.0	33.3±4.2	89.0	23.3±1.2*	119.5
4.	FC *F. redolens*	88.3	88.3	44.2±3.4	118.2	23.6±1.6*	121.0
5.	FC *A. alternata*	90.0	90.0	31.8±2.3	85.0	16.7±1.3	85.6

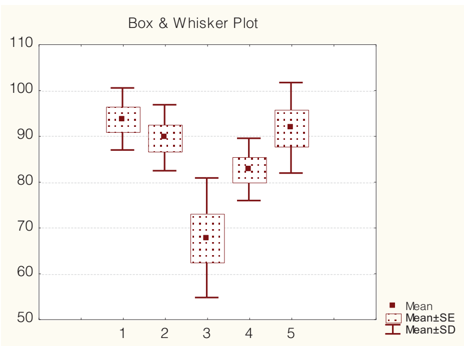
No.	Variant	Germi-nation, %	% relative to control	Length of radicle, mm	% relative to control	Length of stemlet, mm	% relative to control
'Mary Gratefully'							
1.	H_2O (control)	91.7	100.0	43.5±2.7	100.0	26.8±1.3	100.0
2.	FC *F. oxysporum*	92.7	101.1	46.6±92.7	107.2	23.3±1.4	86.9
3.	FC *F. solani*	78.2	85.3	42.0±4.2	96.5	23.3±1.8	86.9
4.	FC *F. redolens*	70.9	77.3	24.0±2.9*	55.2	9.5±1.6*	35.3
5.	FC *A. alternata*	81.8	89.2	11.0±0.8*	25.3	8.6±0.8*	32.1
'Desteptarea'							
1.	H_2O (control)	85	100.0	40.9±2.9	100.0	27.1±1.9	100.0
2.	FC *F. oxysporum*	76.5	90.0	13.6±1.5*	33.2	12.7±1.7*	46.9
3.	FC *F. solani*	80.4	94.6	21.6±3.0*	52.8	23.8±2.3	87.8
4.	FC *F. redolens*	80.4	94.6	56.3±3.3*	137.6	28.2±1.8	104.1
5.	FC *A. alternata*	100.0	117.6	53.6±3.9*	131.0	26.6±1.7	98.1
'Milenium'							
1.	H_2O (control)	86.7	100.0	36.9±2.8	100.0	22.9±1.4	100.0
2.	FC *F. oxysporum*	98.1	113.1	13.9±1.0*	37.7	8.9±0.8*	38.9
3.	FC *F. solani*	73.1	84.3	14.0±2.1*	37.9	13.8±3.7*	60.3
4.	FC *F. redolens*	84.6	97.6	27.3±2.7*	74.0	17.2±1.6*	75.1
5.	FC *A. alternata*	100.0	115.3	26.1±2.5*	70.7	15.7±1.5*	68.6

For example, estimating the capacity of seeds germination of approved varieties after treating them with FC of mentioned above fungi we found that varieties 'Exclusiv', 'Desteptarea', 'Milenium' manifested the highest resistance. This index has diminished under the action of these pathogens to 6.9-27.6%; 5.4-10.0%; 2.4-15.7%, respectively, but sometimes significant stimulation was recorded.

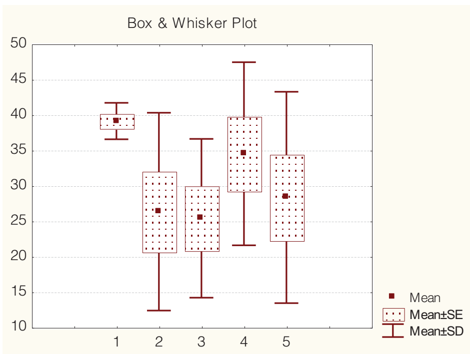
Thus, the variety 'Milenium' under the influence of FC *F. oxysporum* demonstrated increasing 13.1%, 'Desteptarea' and 'Milenium' under the influence of FC *A. alternata* – 17.6 and 15.3%, respectively.

The highest complex resistance was recorded at the 'Mihaela' variety by the evaluation of the reaction of radicles and stemlets to the action of FC of four fungi used in research, the phenomenon was manifested as insignificant diminishing or stimulation: -15.0 ... + 18.2% and -14, 4 ... + 21.0%, respectively, for radicles and stemlets.

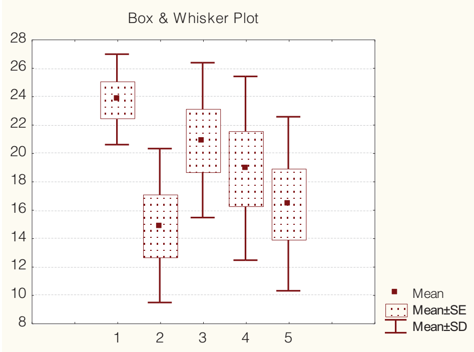
In order to elucidate the peculiarities of action of fungi *F. oxysporum, F. solani, F. redolens* and *A. alternata* on the capacity of germination, growth of radicle and stemlet in tomato the analysis Box & Whisker Plot was proceeded, 6 varieties were used as cases (Figure 1).



Germination, %



Length of radicle, mm



Length of stemlet, mm

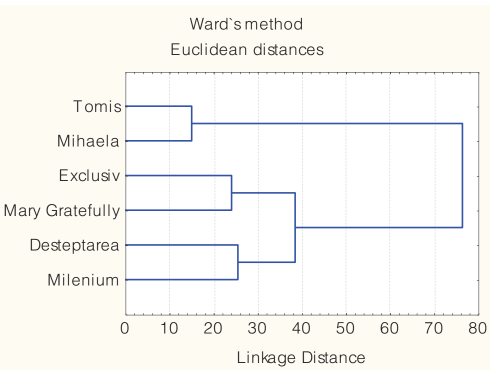
Figure 1. The influence of culture filtrates of causative agents of root rot in tomato to the growth and development of seedlings at the early stages:
1 – control (H_2O), 2 – FC *F. oxysporum*, 3 – FC *F. solani*, 4 – FC *F. redolens*, 5 – FC *A. alternata*

The data obtained showed that in the case of germination the strongest repression (with statistical support, $p \leq 0.05$) was recorded under the influence of fungus *F. solani* (-27.7%) and *F. redolens* (-11.7%), in the case of radicle growth – *F. solani* (-35.1%), and in the case of stemlet growth – *F. oxysporum* (37.4%) and *A. alternata* (-31.0%). The results demonstrated the specificity of action of these pathogenic fungi on growth and development of tomato seedlings at the early stages of ontogeny. From such reason we can conclude that the complex test of perspective tomato forms is necessary in the process of creation of varieties which are capable to develop normal growth organs in the presence of mentioned fungi in the soil.

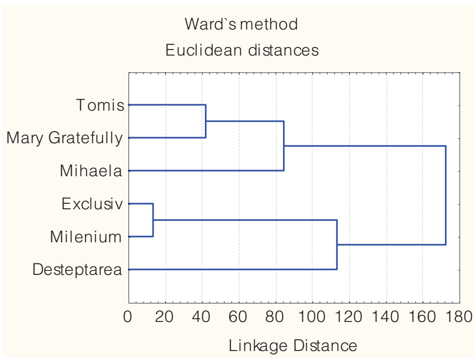
By cluster analysis some similarities and differences between studied varieties were found when the reaction of germination and growth of seedlings to the influence of fungi *Fusarium* spp. and *A. alternata* was registered (Figure 2).

Thus, similarity of varieties 'Tomis' and 'Mihaela' was shown by analysis of germination, they have diminished indices; 'Exclusiv' and 'Mary Gratefully' – intermediate level; 'Desteptarea' and 'Milenium' – the highest germination and sometimes attested stimulation.

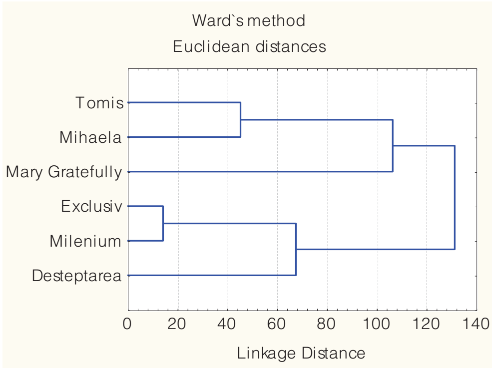
Analyzing growth of radicle and stemlet we found that following variety 'Tomis', 'Mihaela' and 'Mary Gratefully' has demonstrated similarity of these indexes as high. This tells about their fitness for production, provides additional conditions for sowing seeds or seedlings to avoid the danger of reducing their germination capacity in the case of strong soil infestation with causal agents of root rot.



Germination, %



Length of radicle, mm



Length of stemlet, mm

Figure 2. The dendrogram distribution of approved tomato varieties on the basis of similarity of responses to pathogens *Fusarium* spp. and *Alternaria alternata*

By bifactorial analysis of variance it was found that the greatest contribution in the variation of length of radicle embryonic and stemlet had a species of fungus, its contribution was 41.07 and 58.58%, respectively (Table 2).

Table 2. Factorial analysis of the *tomato genotype x fungal pathogen* relationships in tomato

Source of variation	Degree of freedom	Mean sum of squares	Contribution in the source of variation, %
Length of radicle			
Tomato genotype	5	8100*	32.49
Species of fungus	4	10241*	41.07
Tomato genotype x species of fungus	20	6242*	25.03
Random effects	1424	351	1.41
Length of stemlet			
Tomato genotype	5	1210.2*	22.00
Species of fungus	4	3222.3*	58.58
Tomato genotype x species of fungus	20	975.1*	17.73
Random effects	1103	93.5	1.70

*-p≤0,05

It was established that the genotype played an important role too; its factorial pondering consisted of 32.49% and 22.00% for the length of radicle and stemlet, respectively; the interaction *tomato genotype x species of fungus* was recorded as 25.03% and 17.73%, respectively, for both characters. The significant pondering of the contribution of fungus species (*Fusarium* spp., *A. alternata*) and interaction *tomato genotype x species of fungus* in the variation of growth plant organs reveals the necessity of constant monitoring of the composition of species of pathogen agents and their virulence.

The 12 lines obtained by intra-species crosses were studied in order to identify tomato lines with increased resistance to pathogens *Fusarium* spp. on the base of reaction to filtrates of fungi cultures, varieties 'Elvira' and 'Trapeza' were used as the control.

In the control variants the ***germination*** of seeds ranged within 88.3 - 95.0% in most lines excepting two lines L 202 and L 309: 61.7; 65.0% (Table 3).

The results demonstrated that FC of studied fungi influenced different on seeds germination.

For example, inhibition was -5.7 ... -24.2% under the influence of *F. oxysporum* FC. Strong repression was found for 'Trapeza' (-17.0%), L 207 (-22.8%), L 315 (-24.2%), lack of reaction – f or L 202, L 204, L 204, L 206, stimulation: L 208 (+ 9.8%), L 313 (+ 5.3%), L 314 (+ 8.0%), insignificant cases: 'Elvira', L 203, L 308 (-5.7 ... - 9.2%).

In the case of FC *F. solani* inhibition was more pronounced and varied in 1.9 ... 30.5%. Significant inhibition was registered in 'Trapeza' (-30.1%), L 203 (-30.5%), L 308 (23.7%), L 314 (-24.0%), L 315 (-15.5 %), L 207 (-14.0%), lack of reaction in the line L 309, stimulation – L 204 (+ 5.3%), insignificant reaction: L 202 (-2.8%), L 204 (5.3%), L 204 (+ 7.3%), L 206 (-3.3%), L 208 (7.9%), 'Elvira' (-1.9%), L 313 (7, 1%).

FC *F. redolens* produced inhibition at 12 forms from 14, it varied within the ranges -1.9 ... -27.3%, two forms – L 202, L 309 – had no response to the treatment. It should be mentioned that repression was up to 10% in 8 genotypes.

Table 3. The influence of culture filtrates of root rot causative agents to the seeds germination

Line	Origin	Control H_2O	*F. oxysporum*	% relative to control	*F. solani*	% relative to control	*F. redolens*	% relative to control
	Trapeza, control	88.3	73.3	-17.0	61.7	-30.1	75.0	-15.1
202	F_7 Maestro x Irișca	61.7	61.7	0	60.0	-2.8	61.7	0
203	F_6 (Maestro x Irișca) x Maestro	98.3	91.7	-6.7	68.3	-30.5	76.7	-22.0
204	F_6 (Maestro x Irișca) x Irișca	93.3	93.3	0	98.3	+5.3	86.7	-7.1
204a	F_6 (Maestro x Irișca) x Irișca	91.7	91.7	0	85.0	-7.3	85.0	-7.3
206	F_6 (Maestro x D.M.M.) x D.M.M.	100.0	100.0	0	96.7	-3.3	90.0	-10.0
207	F_7 'Mihaela' x Irișca	95.0	73.3	-22.8	81.7	-14.0	83.3	-12.3
208	F_7 'Mihaela' x D.M.M.	85.0	93.3	+9.8	78.3	-7.9	83.3	-2.0
	Elvira, martor	88.3	83.3	-5.7	86.6	-1.9	83.3	-5.7
308	F_{13} Nistru x L 325	91.7	83.3	-9.2	70.0	-23.7	66.7	-27.3
309	F_{11} Nota x Kecskemeti	65.0	55.0	-15.1	65.0	0	65.0	0
313	F_{13} Novicioc x Iuliana	95.0	100.0	+5.3	88.3	-7.1	88.3	-7.1
314	F_{12} Uspeh x L 325	83,3	90.0	+8.0	63.3	-24.0	81.7	-1.9
315	F_{10} Nistru x Saladette	96.7	73.3	-24.2	81.7	-15.5	88.3	-8.7

Radicle embryonic. It was found that genotypes demonstrated very different susceptibility to FC, mean values with the relation to the control varied within the limits -0.4 ...-75.5% for *F. oxysporum* isolate, - 9.6 ... -76.4% – *F . solan*i, and -12.4 ... -42.5% – *F. redolens* (Table 4). It should be mentioned that *F. oxysporum* in 9 cases from 14 had stimulating influence (+3.4 ... + 169.0%), L207 manifested a strong sensitivity (-75.0%). Evaluated lines were most strongly influenced by *F. solani.*

Eleven from 14 genotypes exhibited a suppression which was within the limits -9.6....-76.4%. Stimulation was found in 'Trapeza' (+ 8.4%), L 206 (+ 19.0%) and L 315 (+ 4.5%). In the variant with FC *F. redolens* 7 from 14 genotypes showed inhibition, but 6 lines demonstrated the stimulation of radicle growth. So the lines were strongly different in this analyzed character that reveals the opportunity to identify resistant genotype.

Table 4. The influence of culture filtrates of root rot causative agents to the embryonic radicle length

Line	Origin	Control H_2O	*F. oxysporum*	% relative to control	*F. solani*	% relative to control	*F. redolens*	% relative to control
	Trapeza	40.6±1.88	42.0±1.96	+3.4	44.0±3.58	+8.4	41.0±2.32	+1.0
202	F_7 Maestro x Irişca	27.8±1.91	27.7±2.03	-0.4	9.5±1.18	-65.8	38.2±1.92	+37.4
203	F_5 (Maestro x Irişca) x Maestro	30.3±1.39	35.9±2.00	+18.5	17.3±3.07	-42.9	18.6±1.52	-38.6
204	F_5 (Maestro x Irişca) x Irişca	27.7±1.26	36.8±2.31	+32.8	22.4±2.39	-18.2	32.0±1.97	+15.5
204a	F_5 (Maestro x Irişca) x Irişca	13.6±1.03	36.7±2.38	+169.8	12.3±1.10	-9.6	28.7±1.75	+111.0
206	F_5 (Maestro x D.M.M.) x D.M.M.	40.7±1.88	52.5±1.77	+29.6	48.2±2.15	+19.0	42.6±2.02	+5.2
207	F_7 'Mihaela' x Irişca	37.2±2.05	9.1±0.75	-75.5	15.9±2.45	-57.0	40.82±2.42	+9.7
208	F_7 'Mihaela' x D.M.M.	48.4±2.49	47.2±2.12	-2.5	30.0±3.81	-38.0	30.5±2.14	-37.0
	Elvira	36.0±2.48	47.6±2.42	+32.2	16.5±2.10	-54.2	20.7±2.01	-42.5
308	F_{13} Nistru x L 325	45.1±2.54	54.0±2.14	+19.7	11.8±1.91	-73.8	37.7±2.32	-16.4
309	F_{11} Nota x Kecskemeti	23.8±4.56	23.0±2.24	-3.4	12.9±1.94	-45.8	24.4±3.35	+2.5
313	F_{13} Novicioc x Iuliana	45.9±2.00	48.1±1.95	+4.8	30.7±2.82	-33.1	40.2±2.07	-12.4
314	F_{12} Uspeh x L-325	38.1±1.75	43.1±2.64	+13.1	9.0±0.83	-76.4	22.5±2.04	-41.0
315	F_{10} Nistru x Saladette	35.7±1.71	30.8±2.09	-13.7	37.3±1.83	+4.5	29.7±1.33	-16.8

Stemlet. Its repression varied within the limits -0.5...-74.3% for *F. oxysporum*, -16.7… -74.5% – *F. solani*, -5.2...-56.6% – *F. redolens* (Table 5). Strong sensitivity to *F. oxysporum* was recorded in lines L 207 (-74.3%), L 208 (33.3%), L 202 (-19.2%), L 315 (-26.8%), stimulation was registered in 5 lines: L 203 (+ 5.8%), L 204 (+ 11.1%), L 204a (+ 58.5%), L 308 (+ 23.8%), L 314 (+ 1.6%) (Table 5). Filtrate of culture of *F. solani* in 10 cases from 14 inhibited stemlets growth. High sensitivity was found in lines: L 202 (-74.5%), L 206 (26.1), L 308 (-64.9%), L 309 (-50.0%), L 314 (- 42.8), stimulation in the variety 'Trapeza' (+ 1.4%); L 203 (+ 5.8%), L 204 (+ 47.7%), L 204a (+ 3.8%).

Thus, the lines L 203, L 208 and variety 'Elvira' were the most sensitive to *F. redolens,* repression consisted of 46.8; 56.6 and 40.2%, respectively. Strong stimulation was found in L 309 (52.5%).

Table 5. The influence of culture filtrates of root rot causative agents to the tomato stemlet length

Line	Origin	Control H_2O	*F. oxyspo-rum*	% relative to control	*F. solani*	% relative to control	*F. redolens*	% relative to control
	Trapeza	21.2±1.03	20.2±1.27	-4.7	21.8±1.57	+1.4	25.0±1.72	+17.9
202	F_7 Maestro x Irişca	18.8±1.30	15.2±1.33	-19.2	4.8±1.21	-74.5	20.6±1.32	+9.6
203	F_6 (Maestro x Irişca) x Maestro	18.8±1.06	19.9±1.31	+5.8	19.9±3.51	+5.8	10.0±0.90	-46.8
204	F_6 (Maestro x Irişca) x Irişca	15.3±0.70	17.0±0.95	+11.1	22.6±1.64	+47.7	17.2±1.43	+12.4
204a	F_6 (Maestro x Irişca) x Irişca	13.0±1.17	20.6±1.52	+58.5	13.5±2.94	+3.8	14.0±0.85	+7.7
206	F_6 (Maestro x D.M.M.) x D.M.M.	31.4±1.07	26.6±0.85	-15.5	23.2±1.05	-26.1	23.6±1.39	-24.9

Line	Origin	Control H_2O	*F. oxyspo-rum*	% relative to control	*F. solani*	% relative to control	*F. redolens*	% relative to control
207	F_7 'Mihaela' x Irişca	27.2±1.26	7.3±1.31	-74.3	21.8±3.24	-19.9	25.8±1.56	-5.2
208	F_7('Mihaela' x D.M.M.)	28.8±1.88	19.2±1.01	-33.3	23.3±1.57	-19.1	12.5±1.31	-56.6
	Elvira	20.4±1.38	20.5±1.25	-0.5	17.0±1.83	-16.7	12.2±1.26	-40.2
308	F_{13} Nistru x L 325	26.5±1.25	32.8±1.41	+23.8	9.2±2.78	-64.9	18.8±1.58	-29.1
309	F_{11} Nota x Kecskemeti	11.8±3.16	11.7±1.37	-0.9	5.9±1.45	-50.0	18.0±2.74	+52.5
313	F_{13} Novicioc x Iuliana	28.4±1.04	27.5±1.53	-3.2	22.9±1.61	-19.4	21.2±1.47	-25.4
314	F_{12} Uspeh x L 325	18.7±1.13	19.0±1.34	+1.6	10.7±3.12	-42.8	12.1±1.05	-35.3
315	F_{10} Nistru x Saladette	27.2±0.94	19.9±1.45	-26.8	21.0±0.97	-22.8	20.5±1.25	-24.6

Cluster analysis (*Ward method*) allowed finding similar particularities in created varieties and lines with respect to studied characters: germination, length of radicle and stemlet (Figure 3).

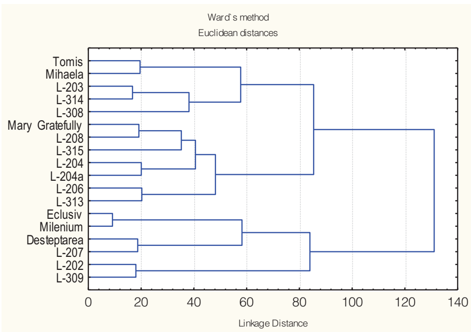


Figure 3. The degree of similarity of tomato varieties and lines on the base of the reaction to pathogens *Fusarium* spp.
(***Cases***: germination, length of radicle, length of stemlet)

Clusters of genotypes with similar response to pathogens *Fusarium* spp. were identified. It was found that the lines L 203, L 208, L 314 were high similar with the varieties 'Tomis' and 'Milenium', and L 202, L 207, L 209 – with varieties 'Exclusiv', 'Milenium' and 'Desteptarea'.

Cluster analysis of *k-means* (*method centroid*) demonstrated that groups of varieties and lines were separated into three clusters on the base of different reaction to FC *Fusarium* spp. (Figure 4).

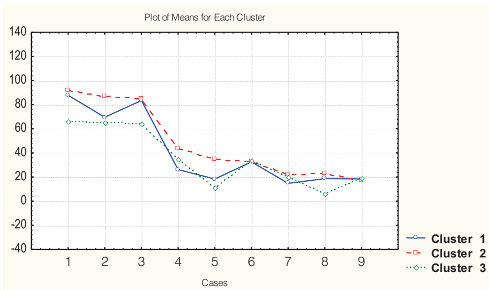


Figure 4. Cluster analysis (*k*-means) of distribution of genotypes on the base of response to culture filtrates of *Fusarium* spp.
Horizontal: *germination* (% to the control) under the FC influence : 1 – *F. oxysporum, 2 – F. solani, 3 – F. redolens; length of radicle*: 4 – *F. oxysporum*, 5 – *F. solani*, 6 – *F. redolens*; *length of stemlet*: 7 – *F. oxysporum*, 8 – *F. solani*, 9 – *F. redolens*.
Vertical: 1, 2, 3 – clusters of genotypes

As members of the *cluster 1* were: 'Tomis', 'Exclusiv', 'Mihaela', 'Desteptarea', 'Milenium', L 203, L 204a, L 207, L 314; *cluster 2:* 'Mary Gratefully', L 204, L 206, L 208, L 313, L 315; *cluster* 3: L 202, L 308, L 309. The varieties of clusters 1 and 2 showed higher resistance compared with the cluster.

CONCLUSIONS

It has been found that the pathogens *Fusarium* spp. and *A. alternata* significantly influenced on the early ontogeny of tomato genotypes by the suppression of seeds germination, growth of radicle and stemlet (sometimes - by their stimulation). Cluster analysis using *k-means* method allowed to find that fungal species *F. solani* and *F. redolens* showed a higher discriminating capacity on varieties / lines of tomato in comparison with *F. oxysporum*. This reveals more pronounced specificity of interaction with these pathogens.

The varieties 'Exclusiv', 'Desteptarea', 'Milenium' manifested the highest resistance in seeds germination capacity, as well as the variety 'Mihaela' demonstrated the highest complex resistance in respect with the reaction of radicle and stemlet to all 4 culture filtrates.

Using factorial analysis it was found that the species of fungus (*Fusarium* spp., *A. alternata*) included the greatest contribution to the source of variation of radicles and stemlets length, its contribution consisted of 41.07 and 58.58%, respectively.

The significant pondering of the influence of fungus species (*Fusarium* spp., *A. alternata*) and the interaction tomato genotype x species of fungus on the source of variation of plant growth organs reveals the necessity of constant monitoring of the composition and virulence of fungal species causing root rot in tomato.

REFERENCES

Amini J., Sidovich D.F., 2010. The Effects of Fungicides on Fusarium oxysporum f. sp. lycopersici Associated with Fusarium Wilt of Tomato. In: J. of Plant Protect. Research, vol. 50, nr. 2, 172-178.

Foolad M.R., 2007. Genome mapping and molecular breeding of tomato. In: International J. of Plant Genomics, 52.

Gavrilescu M., Chisti Y., 2005. Biotechnology – a sustainable alternative for chemical industry. In: Biotechnol. Adv., vol. 23, 471-499.

Manczinger L., Antal Z., Kredics L., 2002. Ecophysiology and breeding of mycoparasitic Trichoderma strains. In: Acta Microbiologica et Immunologica Hungarica, vol. 49, 1-14.

Polixenova V.D., 2009. Induced resistance of plants to pathogens and abiotic stress factors (in the example of tomato). Annals of BSU, Ser. 2, N 1, 48-60.

Savary S. et al., 2010. Use of Categorical Information and Correspondence Analysis in Plant Disease Epidemiology. In: Adv. in Bot. Research, vol. 54, 190-198.

Scott J.W., Gardner R.G., 2007. Breeding for Resistance to Fungal Pathogen. In: Genetic Improvement of Solanaceous Crops. Vol. 2. Tomato. Ed. Razdan M.K., Mattoo A.K., USA, Sci. Publishers, 421-456.

Yamagishi D. et al., 2006. Pathological evaluation of host-specific AAL-toxins and fumonisin mycotoxins produced by *Alternaria* and *Fusarium* species. In: J. of General Plant Pathol., Vol. 72, Issue 5, 323-327.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF TEMPERATURE DATA USAGE THE METHOD OF DEGREE-HOUR IN GREENHOUSES: PEPPER PLANT CASE

Atilgan ATILGAN[1], Ali YUCEL[2], Cagatay TANRIVERDİ[3], Hasan OZ[1], Ahmet TEZCAN[4]

[1]Suleyman Demirel University, Agriculture Faculty, Agricultural Structure and Irrigation Department, 32260 Isparta, Turkey
[2]Osmaniye Korkut Ata University, Osmaniye Vocational School, 80000 Osmaniye, Turkey
[3]Kahramanmaras Sutcu Imam University, Agriculture Faculty, 46100 Kahramanmaraş, Turkey
[4]Akdeniz University, Agriculture Faculty, Agricultural Structure and Irrigation Department, 07100 Antalya, Turkey

Corresponding author email: ctanriverdi@ksu.edu.tr

Abstract

Greenhouses where the production is carried out and optimum environmental conditions achieved according to many plant species are the agricultural structures. Degree-hour or degree-day methods can be used to get knowledge about energy requirements of any buildings. These methods can provide information about on the amount of energy by using measured and meteorological values and obtaining the heating and cooling values of buildings. The study was chosen in the Kumluca district of Antalya, the place where the most intensive of greenhouse cultivation was made in our country. Autumn cultivation is being done for the pepper plant. This study was conducted between October 2015 and June 2016 in plastic greenhouse. The temperature inside the greenhouse values were recorded during the growing period of the pepper plant. The obtained values were analyzed by Student-t test. The regression coefficients were obtained by calculating the prediction equations between the basic temperature values and the heating and cooling degree-hour values. These coefficients are found between 0.997 and 0.999 for heating degrees-hours and between 0.982 and 0.997 for cooling degrees. As a result, efficiency and quality can be increased through air conditioning improvements in the greenhouses located in mild climate areas such as Antalya, where heating is maintained for frost protection and ventilation difficulties are experienced.

Key words: *pepper, greenhouse, degree-hour, temperature.*

INTRODUCTION

Greenhouses are the agricultural production facilities where production is planned based on technical and economic factors with a view to ensuring the right air conditioning and production planning required for the type of plant to be cultivated.

Having a history of more than 50 years, greenhouse cultivation is conducted on nearly 66,000 hectares of land throughout Turkey, primarily in the Mediterranean Region as well as the Aegean, Marmara and Black Sea regions (Olgun, 2011; Anonymous, 2016).

Antalya is the city in the Mediterranean Region where greenhouse cultivation is most extensively conducted. Kumluca county in the province of Antalya, however, provides the biggest greenhouse cultivation. When the cultivation of the pepper plant in Kumluca area is compared to the overall greenhouse cultivation in Turkey in 2015, it is observed that while the entire greenhouse cultivation of peppers in Turkey took place in nearly 1000 hectares of land, the area devoted to the production of peppers in Kumluca accounted for 30% of the entire production area (300 hectares), which represented 34% (36,000 tons) of the total national production of 105,000 tons.

In the same way, of the entire domestic plastic greenhouse cultivation of peppers performed on a land of 3580 hectares, 755 hectares (21%) of it is performed in Kumluca.

Of the 301,600 tons of peppers produced in the plastic greenhouses in Turkey, 90,600 tons

(30%) are produced in Kumluca county (Anonymous, 2016).

Pepper is a plant that is quite fond of light yet insensitive to the duration of daylight. Although it is capable of germinating at above 8°C, the best germination is achieved at 21°C to 28°C.

While the ideal environmental temperature is between 18-23°C, during day and night, when it is a seedling, care should be taken to ensure that night temperature is not below 12°C. While the daily temperature can be allowed to rise to 25°C on sunny days, ventilation should nevertheless be performed when the temperature hits 30°C. Cultivation is ceased fully at 45°C (Sevgican, 1999).

Planning on heating, cooling and ventilation systems in greenhouses are related to the outside air conditions.

While planning such systems, reliance on long term climate data rather than the climate conditions of several years will help planners adopt a more realistic approach in determining future results (Ileri and Uner, 1998). Degree-day values are one of the most basic measurement units used in the estimation of annual energy requirements of a building located in any place or location (Bayram and Yesilata, 2009).

This study aims to identify the relationship between the indoor temperature and the values of heating and cooling degree-hour values in greenhouse cultivation.

To this end, the indoor temperature values measured inside the greenhouse have been calculated and interpreted based on the heating and cooling durations during the cultivation period of peppers through degree-hour method.

MATERIALS AND METHODS

This study was conducted in Kumluca county, Antalya, where pepper production is extensively performed, in a 4 x 19.5 x 62 m size plastic greenhouse (Figure 1).

It was conducted during the dates between October 2015 and June 2016, a period considered to be the cultivation period of peppers. The temperature was measured by using four TESTO 175 H1 brand temperature and moisture meter sensor.

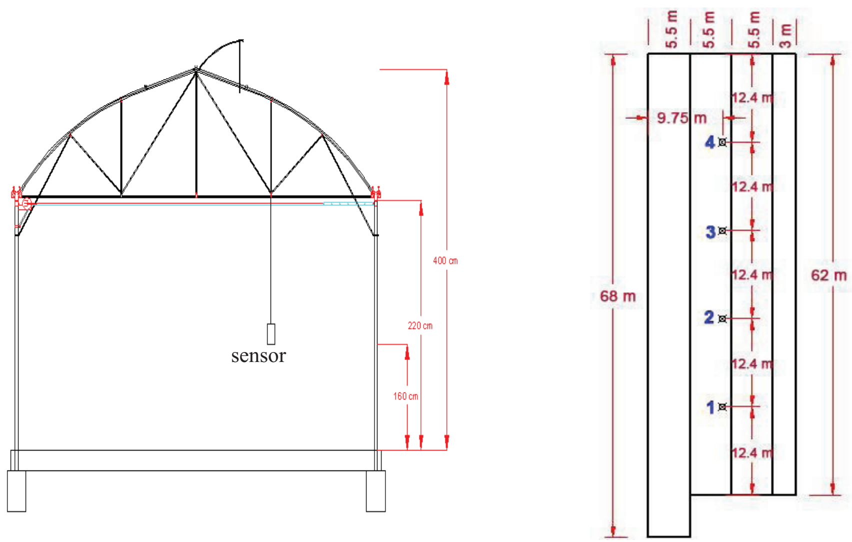


Figure 1. View of the sensors and plastic greenhouse that used in this study

Degree-hour method

Degree-hour and degree-day methods are used in the calculation of heating and cooling loads of structures and air conditioning systems. Degree-hour method yields more precise results than degree-day method (Pusat et. al., 2014).

In the degree-hour method, the energy required for heating or cooling a structure is in proportion with the difference between the air temperature and the balance point temperature. The heating process will be required when the air temperature (Ta) drops below the balance point temperature (Tb). The cooling process will be required when the air temperature (Ta) rises over the balance point temperature (Tb). Heating degree-hour (HDH) and cooling degree-hour (CDH) values can be calculated based on the following equations (Buyukalaca et al., 2001; Bulut et al., 2007; Pusat et al., 2014; Pusat et al., 2015).

$$HDH = (1hour)\sum_{i=1}^{n}(T_b - T_a)^+ \qquad (1)$$

$$CDH = (1hour)\sum_{i=1}^{n}(T_a - T_b)^+ \qquad (2)$$

Here; T_a represents the ambient temperature (°C), T_b the balance point temperature (°C), n the hours of the year, the mark in the equations above suggests that only the positive values will be used.

In the calculation of heating and cooling hours of the pepper plant, the temperature values for autumn cultivation was taken as the basis (Table 1) (Ozalp et al., 2006; Anonymous, 2016).

Table 1. Suggested temperature values for autumn period of pepper plant

Growth period	Suggested temperatures	Date	Period
Greenhouse planting	22-28 ^oC	The end of September- Beginning of October	7-14 days
Flower formation, pollination, insemination	20-25 ^oC	2^{nd} of October week - 4^{th} of October week	5-6 weeks
Fruit ripening	16-25 ^oC	2^{nd} of November week -4^{th} of of December week	6 weeks
Harvest	20-35 ^oC	2^{nd} of February week- 1^{st} of May week	10 weeks

RESULTS AND DISCUSSIONS

The heating degree-hour graphs are presented in the Figure 2 by taking into account the recommended temperature values for each cultivation period of the pepper plant (planting, flower formation**,** pollination, insemination, fruit ripening and harvest). The graphs suggest that the heating degree-hour values increase linearly in accordance with the increasing temperature requirement of the pepper plant in every period. In their study, Bulut et al. (2007) have pointed out that the heating and cooling day values increase linearly depending on the basic temperature values.

The linear increase observed in this study concurs with the findings of Bulut et al. (2007). According to the temperature values measured by the sensors during each cultivation period inside the greenhouse, the calculated numbers of HDHs are found to be fairly close to one another.

It can be argued that there is a homogenous temperature distribution maintained inside the greenhouse. Because heating is maintained in mild climates only for the purpose of protecting the plant against frost and thus heaters are used (Büyüktas et al., 2016). In the greenhouse where the present study was conducted heating was maintained through heaters and it was stated that this practice was exclusively intended for protecting the plant against frost. Availability of HDH values at every heating requirement throughout each cultivation period of the pepper plant and the fact that they follow a different course than that of the recommended temperature values suggest that the greenhouse was not being heated sufficiently.

Insufficient heating in a greenhouse, on the other hand, leads to a significant decrease in both yield and quality (Sevgican, 1999).

The graphs provided herein present the equations and regression coefficients of different base temperature values recommended throughout the cultivation period (independent variable) and the HDH values (dependent variable).

Upon reviewing such coefficients, it has been established that the regression coefficient (R^2) between the dependent and independent variables vary between 0.996 and 0.999.

This can be explained by the presence of a highly positive relationship.

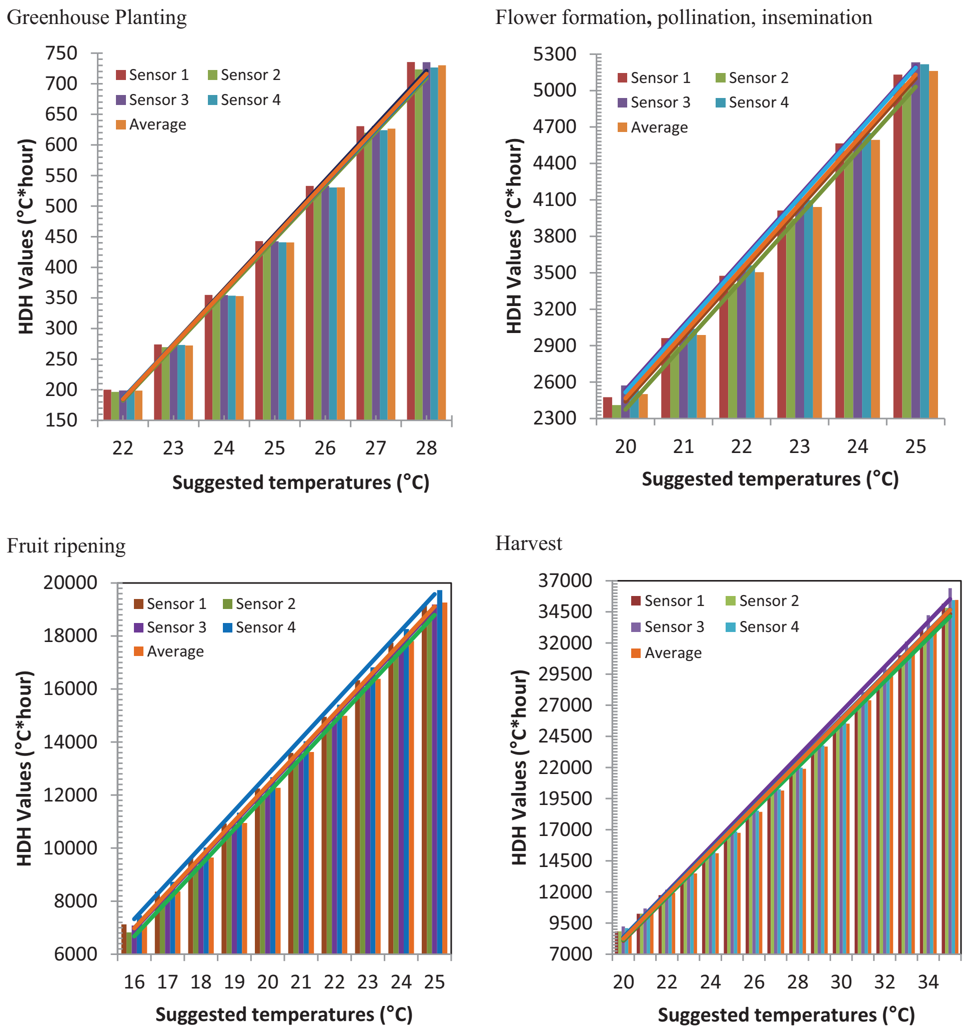


Figure 2. Heating degree-hour values dependent on different temperature values for the pepper plant

The CDH values of the pepper plant have been calculated and presented in the Figure 3.

The CDH values were found to be at a maximum of the values where the heating requirement is reduced for the pepper plant at each cultivation period.

The heating requirement of 22°C during planting, 20°C during florescence, 16°C during fruit development and 20°C during harvesting were determined as the maximum CDH values. The greenhouse, in its current condition, is capable of providing such temperature values. The fact that the daytime temperature values are higher than that of the night time temperature can be explained by the availability of the CDH values even at the minimum temperature requirements required for the pepper plant.

In their study, researchers (Bayram and Yesilata, 2009; Yucel et al., 2014) state that the numbers of heating degree-days and cooling degree-days are important in terms of determining the capacity and costs of the heating and cooling systems.

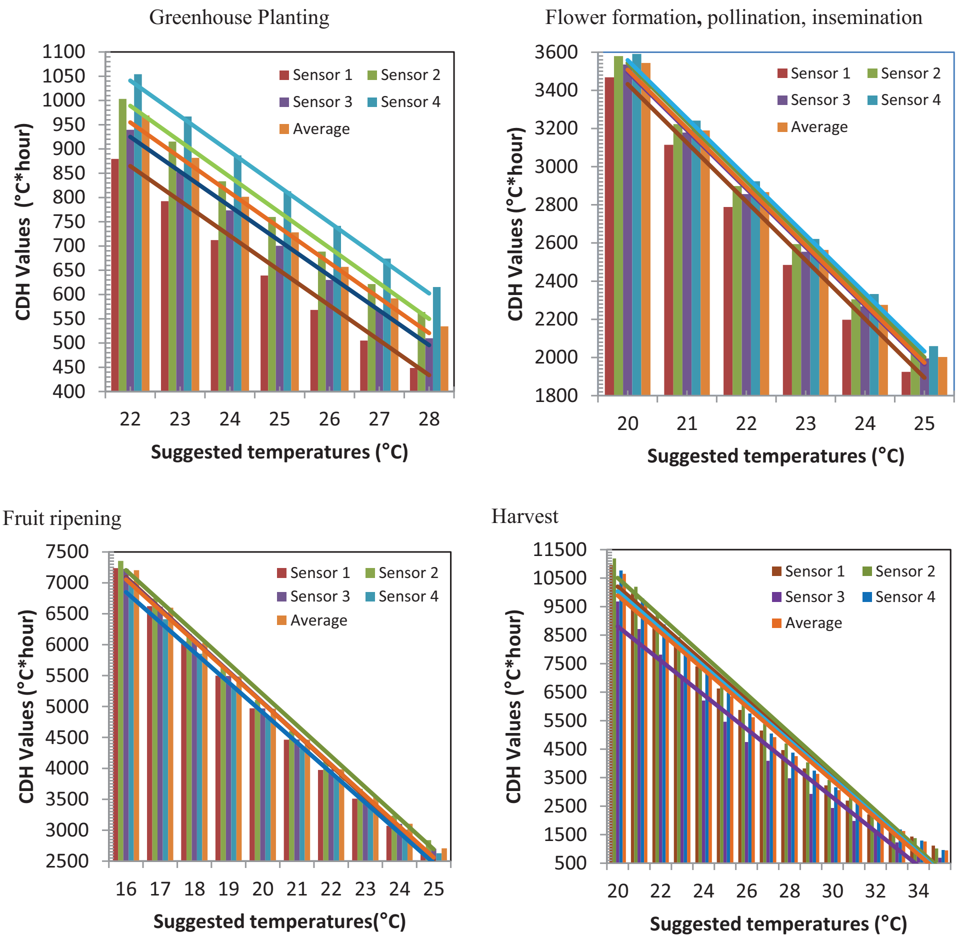


Figure 3. Cooling degree-hour values dependent on different temperature values for the pepper plant

It is reported that the biggest problem encountered in plastic greenhouses is the issue of ventilation. In their study, Monetero et al. (2001) and Fatnassi et al. (2009) report that there are serious efficiency and quality related issues in the greenhouses located around the Mediterranean area, especially at the beginning of the spring and end of the autumn, due to high indoor temperature and insufficient ventilation.

Despite the ventilation provided through both the side walls and the roof, the greenhouse in which the present study was conducted was found to be insufficiently cooled based on the CDH values.

This can help explain the higher cooling degree-hour values even when the heating is least required with the rise in the outside temperature and in line with the cultivation periods of the pepper plant. Again, the equation and regression coefficients of the dependent and independent variables obtained based on the Figure 3 are presented. It is established that such coefficients (R^2) vary between 0.972 and 0.998.

This can also be explained by the presence of a highly positive relationship.

Based on the heating and degree-hour values, we can argue that the plastic greenhouse where this study has been conducted has failed, in its present condition, to provide the temperature values required for the cultivation of peppers at an optimum level. The recommended temperature value of the pepper plant at any cultivation period can be explained by the presence of both heating and cooling values.

CONCLUSIONS

This study has been conducted in a plastic pepper cultivation greenhouse in Kumluca, Antalya where pepper cultivation is extensively performed. Heating and cooling degree-hour values have been determined as per the recommended temperature values for each cultivation period of the pepper plant. Both the heating and cooling degree-hour values were found to have varied based on the temperature requirement of the pepper plant. It was ascertained from the greenhouse growers in the area that the heating was maintained mostly for the purpose of protecting the plant against frost, and it was verified based on the heating and cooling degree-hour values that both heating and cooling was insufficiently maintained for each cultivation period of the pepper plant due to ventilation problems encountered particularly in block plastic greenhouses. In conclusion, yield and quality can be increased through air conditioning improvements in the greenhouses located in mild climate areas such as Antalya, where heating is maintained for frost protection and ventilation difficulties are experienced. Moreover, the fluctuation in the heating and cooling degree-hour values depending on different cultivation periods of the pepper plant may give an idea to the manufacturers beforehand in terms of energy consumption or use.

REFERENCES

Anonymous, 2016. Pepper Growing (*Capsicum annum* L.). http://www.bahcenet.com/biber-yetistiriciligi-capsicum-annum-l.html. (In Turkish)

Bayram M., Yesilata, B., 2009. Integration of number of heating and cooling degree days, IX. National Plumbing Engineering Congress, May 6-9, 2009, Izmir, 425-432. (In Turkish)

Bulut, H., Buyukalaca, O., Yilmaz, T., 2007. Analysis of Heating and Cooling Degree-hour Values for the Mediterranean Region, 2. National Congress of Air conditioning, 111-122.

Buyukalaca, O., Bulut, H.,Yilmaz, T., 2001. Analysis of variable-base heating and cooling degree-days for Turkey, Applied Energy, 69(4):269-283.

Büyüktas, K., Atilgan, A., Tezcan, A., 2016. Agricultural Production Structures, Suleyman Demirel University, Faculty of Agriculture, Publication no: 101, 251. (In Turkish)

Fatnassi, H., Leyronas, C., Boulard, T., Bardin, M., Nicot, P., 2009. Dependence of greenhouse tunnel ventilation on wind direction and crop height. Biosystems Engineering, 103: 338-343.

Ileri, A., Uner, M., 1998. Typical climate data for Turkish cities, IV. National Installation Engineering Congress and Exhibition, 463: 31-42. (In Turkish)

Montero J.I, Hunt, G.R, Kamaruddin, R., Anton, A., Bailey, B.J., 2001. Effect of ventilator configuration of wind-driven ventilation in a crop protection structure for the tropics. Journal of Agricultural Engineering Research 80:99–107.

Olgun, M., 2011. Agricultural Structures, Ankara University publications No:244, 445p. (In Turkish)

Ozalp, R., Çelik, I., Coskun, A., 2006. Covering pepper growing. Sound of agriculture, March 2006, 9:18-21. (In Turkish)

Pusat, S., Ekmekci, I., Dundar, A.C., Ermis, K., Sen, Y., 2014. Typical meteorological year and degree-hour account for Istanbul, 2. National Symposium on Heating-Cooling Education and Exhibition, 23-25 October 2014, 5, Balıkesir. (In Turkish)

Pusat, S., Tunc, N., Ekmekci, I., Yetisken, Y. 2015. Degree-Time calculations for Karabük, ISITES-2015 Valencia-Spain, Academic Platform, 898-905. (In Turkish)

Sevgican, A., 1999. Protected Cultivation, University of Ege, Agriculture Publications Publ, No: 528, 302p. (In Turkish)

TUİK, 2016. Turkish Statistical Institute, www.tuik.gov.tr (In Turkish)

Yucel, A., Atilgan, A., Oz, H., Saltuk, B., 2014. The determination of heating and cooling day values using degree-day method: Tomato plant example. Infrastructure and Ecology of Rural Areas. Nr VI/I, 1049-1061.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOME NUTRIENT CHARACTERISTICS OF GOLDENBERRY (*PHYSALIS PERUVIANA* L.) CULTIVAR CANDIDATE FROM TURKEY

Aysun OZTURK[1], Yasin ÖZDEMİR[1], Barış ALBAYRAK[2], Mehmet SİMŞEK[3], Kutay Coşkun YILDIRIM[3]

[1]Atatürk Horticultural Central Research Institute, Department of Food Technologies, Yalova, Turkey
[2]Atatürk Horticultural Central Research Institute, Land and Water Resources, Yalova, Turkey
[3]Atatürk Horticultural Central Research Institute, Vegetables Production Department, Yalova, Turkey

Corresponding author email: ozturkaysun@hotmail.com

Abstract

This study was the first record on nutrient characteristics of goldenberry types in Turkey. In this study; 6 types of goldenberry were used as material which were collected from different side of Turkey and cultivated in observation parcel of Atatürk Horticultural Central Research Institute Yalova (Turkey). Obtained data will be used for future selection and registration steps of these types. For this purpose carotene, vitamin C, crude fiber, total phenolic and mineral matter content and antioxidant activity of goldenberries were determined. The results showed that goldenberry types are rich in vitamin C, total phenolic content and minerals (especially phosphorus) content. Also they have been found as a rich source of crude fiber and carotene. 6th type has the highest total phenol (48.60 mg GAE/100g), crude fiber (4.10%) and vitamin C (34.86 mg AAE/100g) content and 3rd type has the lowest total phenol (42.16 mg GAE/100g), vitamin C (31.40 mg AAE/100g) and carotene (1.98 mg/100g) content. According to evaluation of these results 6th type was determined as featured types for registration as new cultivar.

Key words: *Physalis peruviana L., carotene, crude fiber, total phenolic content, mineral matter*

INTRODUCTION

Physalis peruviana L., belongs to the *Solanaceae* family and *Physalis* genus, commonly known as goldenberry or cape gooseberry in English speaking countries. Different varieties of *P. peruviana* are recognized in many countries and are cultivated commercially in tropical and subtropical countries such as South Africa, America, New Zealand and Spain (Morton, 1987).
They are grown in warm climates region of Turkey, especially Black Sea, Marmara, Aegean and Mediterranean regions (Besirli, 1998).
Generally, the fruit of goldenberry is consumed fresh or dried like raisin. It is also used in sauces, glazes for meats, seafood (NRC, 1989). Currently, there are different products processed from goldenberry fruits such as juice, pomace jams, chocolate-covered candies, other products sweetened with sugar as a snack (Ramadan and Moersel, 2009). In European markets, it is used as ornaments in meals, salads, desserts and cakes (Puente et al., 2010).
The fruits of goldenberry were reported to have many medicinal properties such as antispasmodic, diuretic, antiseptic, sedative, analgesic, helping to fortify the optic nerve, throat trouble relief, elimination of intestinal parasites and amoeba (CCI, 1994).
Studies indicated that eating the fruit of goldenberry reduces blood glucose after 90 min postprandial in young adults, causing a greater hypoglycaemic effect after this period (Rodríguez & Rodríguez, 2007).
The fruit of goldenberry has been stated as a rich source of carotene and its major components are its high amounts of polyunsaturated fatty acids, vitamins A, B and C and phytosterols, as well as the presence of essential minerals, vitamins such as E and K_1, with anolides and physalins, which together would give them medicinal properties described above (Ombwara, 2004; Puente et al., 2010).

The aim of this study was to investigate some chemical properties and nutrient composition of goldenberry fruit which were collected from different side of Turkey and cultivated in Atatürk Horticultural Central Research Institute Yalova (Turkey). Obtained data was used for future selection and registration steps of these types. With this study; main features of goldenberry which new known for the food industry and consumers of Turkey were determined.

MATERIALS AND METHODS

Fruits of 6 types of goldenberry were used as material. They were collected from different side of Turkey, numbered from 1 to 6 and cultivated at same conditions in Atatürk Horticultural Central Research Institute.

Figure 1. Goldenberry fruits

Mineral Matter Analysis. Golderberry samples were dried, milled and analyzed according to method of wet decomposition by using sulfuric acid + hydrogen peroxide solution according to Anonymous (1980). Amounts of K, Ca, Mg, Fe, Cu, Zn and Mn elements were determined with atomic absorption spectrophotometer (Perkin-Elmer AAnalyst 700, USA). P element was calculated as colorimetric with vanadomolibdofosforic acid method (Lott et al., 1956).

Ascorbic Acid Analysis. Official spectrophotometric method was used according to AOAC 1970.

Crude Fiber Analysis. Samples were boiled with acid (1.25% H_2SO_4), then alkaline (1.25% NaOH). Later, the residues were burned in an ash furnace (AOAC, 1970).

Extraction of samples for Amount of Total Phenolic Compounds and Antioxidant Activity Analysis: 3 g samples were weighed fresh goldenberry fruits and mixed with 25ml methanol then homogenized for 2 min. and homogenates were kept at +4 °C for 12 h. Samples were centrifuged at 15,000 rpm for 20 min. and liquid phases in methanol were collected with Pasteur pipettes into amber bottles then were stored at -20 °C until analysis. These extracts prepared were used both antioxidant activity and amount of total phenolic compounds (Thaipong et al., 2006).

Amount of Total Phenolic Compounds Analysis. Amount of total phenolic compounds was determined by the Folin - Ciocalteu method. The 150 µl of extract, 2400 µl of distilled water, and 150 µl of 0.25 N Folin - Ciocalteu reagent were combined in a plastic vial and then mixed well using a Vortex. The mixture was allowed to react for 3 min then 300 µl of 1N Na_2CO_3 solution was added and mixed well. The solution was incubated at room temperature (23°C) in the dark for 2 h. The absorbance was measured at 725nm using a Hitachi U-2900 brand spectrophotometer (Tokyo, Japan) and the results were expressed in gallic acid equivalents (GAE mg/100 g) using a gallic acid (0–0.1 mg/ml) standard curve (Thaipong et al., 2006).

Antioxidant Activity Analysis. The DPPH (2,2-difenil-1-pikrilhidrazil) assay was done according to the method of Thaipong et al. (2006). The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and then stored at -20°C until needed. The working solution was obtained by mixing 10mL stock solution with 45 ml methanol to obtain an absorbance of 1.1±0.02 units at 515 nm using the spectrophotometer. Mushroom extracts (150 µl) were allowed to react with 2850 µl of the DPPH solution for 24 h in the dark. Then the absorbance was taken at 515 nm. The standard curve was linear between 25 and 800 µM Trolox. Results were expressed in µM TE/g.

RESULTS AND DISCUSSIONS

Reducing sugar rates of goldenberry types were determined between 6.55 and 7.80 % (Table 1). In a similar study; Sharoba and Ramadan (2010) reported that reducing sugar rates 8.23% in goldenberry fruit.

In our study, the carotene contents of the goldenberry types were found very close to each other. These values were between 1.98-2.30 mg / 100 g (Table 1). Ombrawa (2004) and Sharoba and Ramadan (2010) reported carotene contents respectively as 1.61 and 2.38 mg/100g.

According to Table 1, ascorbic acid amounts of goldenberry samples have showed variability between 35.10-31.40 mg/100 g. Ascorbic acid amounts were given 43.0 mg/100 g in study done by NRC (National Research Council, 1989) and 26,00 mg/100 g in study done by CCI (Corporación Colombia Internacional, 1994) and also 20,00 mg/100 g by Fischer et al.(2000).

The highest crude fiber ratio was calculated as 4.10 % in 6th type goldenberry sample, while the lowest crude fiber ratio was calculated as 3.18 % as in 1th type goldenberry sample (Table 1). Other researchers reported crude fiber ratios as 4.9 % (NRC, 1989); 4,8 % (CCl,1994) and 3.6 % (Repo Depo de Carrasco and Zelada, 2008).

Table 1. Reducing sugar, carotene, ascorbic acid and crude fiber content of goldenberry types

Types	Reducing Sugar (%)	Carotene (mg/100g)	Ascorbic Acid (mg/100g)	Crude Fiber (%)
1	6.55	2.10	33.90	3.18
2	6.79	2.26	33.15	3.24
3	7.80	1.98	31.40	3.85
4	7.20	2.24	35.10	3.94
5	6.21	2.20	32.54	4.02
6	7.75	2.30	34.86	4.10

Amounts of total phenolic compound (TPC) for goldenberry are showed in Table 2. As a result of the analysis, 6th type has the highest TPC amount (48.60 mg GAE/100 g) and 3rd type the lowest TPC (42.16 mg GAE/100 g). In a study conducted by Restrepo (2008), amount of TPC has been specified 40,45±0,93 mg GAE/100 g; in another study has been reported as 39,15±5,43 mg GAE/100 g (Botero, 2008). In terms of TPC amounts, our results have been showed compatibility with literature values.

Antioxidant activity of goldenberry types were determined between 604.69 - 514.06 TEμM/100 g (Table 2). Both Restrepo (2008) and Botero (2008) carried out DPPH method in their studies and they stated antioxidant activity of goldenberry fruit as 210.82±9.45 and 192.51±30.13 TEμM/100 g, respectively. When Table 2 is examined, it was seen that our results are higher than results of Restrepo (2008) and Botero (2008).

Table 2. Amounts of total phenolic compound and antioxidant activities of goldenberry types

Types	Amount of Total Phenolic Compounds (mg GAE/100g)	Antioxidant Activity (TEμM/100g)
1	46.91	566.27
2	47.67	554.69
3	42.16	514.06
4	48.39	604.69
5	48.53	552.08
6	48.60	569.24

Mineral matter amounts of types were found as N 1160-1460 mg/100 g, P 178,46-233,49 mg/100 g, K 1794,98-19,68 mg/100 g, Ca 34,12-43,65 mg/100 g, Mg 102,50-122,51 mg/100 g, Fe 3,68-4,09 mg/100 g, Mn 0,59-0,78 mg/100 g, Zn 1,78-2,32 mg/100 g, Cu 2,19-3,28 mg/100 g, calculated on the basis of total dry matter (Table 3).

In a study carried out in Colombia, mineral matter amounts of goldenberry fruits (on the basis of fresh weight) were informed as: P 27 mg/100 g, K 467 mg/100 g, Ca 23 mg/100 g, Mg 19 mg/100 g, Fe 0,09 mg/100 g, Mn 0,20 mg/100 g, Zn 0,28 mg/100 g, Cu 64 mg/100 g, Na 6 mg/100 g, Cl 1 mg/100 g, S 10 mg/100 g, Ni 0,02 mg/100 g (Leterme et al., 2006). In others studies also were reported: P 55,3 mg/100 g, Ca 8,0 mg/100 g, Fe 1,23 mg/100 g (Ombwara, 2004); P 34 mg/100 g, K 210 mg/100 g, Na 2 mg/100 g, Ca 28 mg/100 g, Fe 0,3 mg/100 g, Mg 7 mg/100 g (Musinguzi et al., 2007).

Amounts differences of mineral matter in between our research and literature have been caused by different climate and growing conditions of the goldenberries.

Table 3. Mineral matter of goldenberry types (mg/100 g dry matter)

Types	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
1	1160	214.91	1911.99	37.37	102.92	3.76	0.59	1.88	2.19
2	1200	217.61	1968.77	40.55	103.13	3.68	0.60	1.78	2.44
3	1220	178.46	1801.85	34.12	102.50	3.72	0.65	1.99	2.88
4	1460	233.49	1794.98	43.65	122.51	4.09	0.78	2.32	2.97
5	1325	222.31	1903.15	41.39	107.82	4.04	0.65	2.14	3.28
6	1455	220.62	1954.12	42.54	115.36	4.02	0.62	2.18	3.08

CONCLUSIONS

Plant phytochemicals especially phenolic compounds are thought to protective cells against oxidative damage and aging effects etc. In this study fruits of goldenberry types are detected as rich source for vitamin C, carotene and phenolic compounds. This study was the first record on nutrient characteristics of goldenberry types in Turkey. According to the analysis results, it was seen remarkably that goldenberry fruit is a good source crude fiber and carotene, furthermore it have low sugar content. 6th type has the highest total phenol, crude fiber and vitamin C content. According to evaluation of results 6th type was considered proper to select and stand out for registration and certification as a new cultivar.

ACKNOWLEDGEMENTS

This research work was carried out Food Technology Department with the support of Atatürk Horticultural Central Research Institute. It also was financed Republic of Turkey Ministry of Food, Agriculture and Livestock. (The project number is TAGEM/HSGYAD/12/A05/P03/14).

REFERENCES

Anonymous, 1970. Association of official analytical chemists (AOAC), Horwitz W, Chichilo P, Reynolds H. Washington, syf 129-131.

Anonymous, 1980. Soil and Plant Testing and Analysis as a Basis of Fertilizer Recommendations. F.A.O. Soils Bulletin 38/2, 95.

Beşirli G., Sürmeli N., 1998. Agriculture Village Journal.

Boter, A., 2008. Aplicación de la Ingeniería de Matrices en el desarrollo da la uchuva mínimamente procesada fortificada con calcio y vitaminas C y E. Facultad de química farmacéutica, vol. Magíster en ciencias farmacéuticas énfasis en alimentos. p185, Medellín: Universidad de Antioquía.

Corporaciòn Colombia International (CCI), Universidad De Los Andes & Departamento De Planeaciòn Nacional, 1994. Análisis internacional del sector hortofrutícola para Colombia. Bogotá: El Diseño

Fischer G., Ebert G., Ludders P., 2000. Provitamin A carotenoids, organic acids and ascorbic acid content of cape gooseberry (*Physalis peruviana* L.) ecotypes grown at two tropical altitudes. Acta Horticulturae, 531, 263−268.

Leterme P., Buldgen A., Estrada F., London A.M., 2006. Mineral content of tropical fruits unconventianal foods of the Andes and the rain forest of Colombia. Food Chemistry 95, 644-652.

Lott W.L., Gallo J.P., Medaff J.C., 1956. Leaf Analysis Technic in Coffee Research. Ibec. Research Institute II: 21-24.

National Research Council (NRC), 1989. Goldenberry (Cape Gooseberry). Lost crops of the incas: Little-known plants of the andes with promise for worldwide cultivation. Washington D.C., National Academy Press, 240−251.

Morton, J. F. Cape gooseberry. In Fruits of Warm Climates; Morton, J. F., Ed.; Creative Resource Systems: Winterville, NC, 1987; pp 430-434.

Musinguzi E., Kikafunda J., Kiremire B., 2007. Promoting indigenouswild edible fruitsto complement roots and tuber crops in alleviating vitamin A deficiencies in Uganda, Arusha, Tanzania: Proceedings of the 13th ISTRC Symposium, 763–769.

Ombwara F.K., Wamocho L.S., Mugai E.N., 2004. The Effect of Nutrient Solution of golStrength and Mycorrhizal Inoculation on Anthesis in *Physalis peruviana*. Proceedings of the fourth workshop on sustainabla hortıcultural production in the tropics. 24-27 Nowember, 117-122.

Puente L. A., Claudia A P., Eduardo S.C., Misael C., 2010. *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review. Food Research International.

Ramadan M.F., Moersel J.T., 2007. Impact of enzymatic treatment on chemical composition, physicochemical properties and radical scavenging activity of goldenberry (*Physalis peruviana* L.) juice. J. Sci. Food Agric. 87, 452–460.

Ramadan M., Moersel J.T., 2009. Oil extractability from enzymatically treated goldenberry (*Physalis peruviana* L.) pomace: Range of operational variables. International Journal of Food Science & Technology, 44(3), 435−444.

Repo Depo De Carrasso R., Zelada C., 2008. Determinación de la capacidad antioxidante y compuestos bioactivos de frutas nativas peruanas. Revista de la Sociedad Química Perú, 74(2), 108−124.

Restrepo A., 2008. Nuevas perspectivas de consumo de frutas: Uchuva (*Physalis peruviana* L.) y Fresa (*Fragaria vesca* L.) mínimamente procesadas fortificadas con vitamina E. Facultad de Ciencias Agropecuarias, vol. Magíster en ciencia y tecnología de alimentos, Medellín: Universidad Nacional de Colombia, 107.

Rodríguez S., Rodríguez E., 2007. Efecto de la ingesta de Physalis peruviana (aguaymanto) sobre la glicemia postprandial en adultos jóvenes. Revista Médica Vallejiana, 4(1), 43−52.

Ross A.F., 1959. Dinitrophenol method for reducing sugar Patato Processing. (Ed: W.F. Talburt). The AVI Publishing Com. İnc., Wesport, Connecticut. 469-470.

Sharoba A.M., Ramadan M.F., 2010. Rheological behavior and physicochemical characteristics of goldenberry (*Physalis peruviana*) juice as affected by enzimatic treatment. Journal of Food Processing and Preservation.

Thaipong K., Boonnprakob U., Crosby K., Cisneros-Zevallos L., Byrne D. H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19, 669–675.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

NEW GENOTYPES OF EGGPLANTS OBTAINED AT V.R.D.S. BUZĂU

Camelia BRATU[1], Florin STĂNICĂ[2], Costel VÎNĂTORU[1], Viorica LAGUNOVSCHI[2], Bianca ZAMFIR[1], Elena BĂRCANU[1]

[1]Vegetable Research and Development Station Buzău, No. 23, Mesteacănului Street, zip code 120024, Buzău, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: botea_camelia2007@yahoo.com

Abstract

The V.R.D.S. Buzău breeding laboratory has put a great emphasis on maintaining the biodiversity of this species by constituting a valuable germplasm collection which has 84 genotypes and it is still growing. Our unit, V.R.D.S. Buzău, has patented so far two varieties, 'Dragaica' and 'Zaraza', and recently the F1 hybrid Rebeca. After evaluating their genetic stability it was found that 36 genotypes are stable, 23 are in an advanced form of stabilization and 25 accessions are still segregating. Researches completed until now by stabilizing an important number of valuable accessions with distinct phenotypic expressivity: A 10 with white fruits, A25A with brindle markings of white and purple, A 26 has purple fruits, A20 with small egg-shaped fruits, A29A. A29B, A29C and A29D with small red fruits at the physiological maturity, arranged in raceme, and of different shapes: round, ovoid, pumpkin etc. From all this varieties, the A10 accession was proposed for patenting and homologation, and will be followed by other varieties that are still under evaluation.

***Key words**: breeding, varieties, Rebeca F1.*

INTRODUCTION

Our country has favorable pedo-climatic conditions for cultivating the *Solanum melongena* species. Preoccupations in breeding this species have been since V.R.D.S. Buzău was founded. For starters, imported varieties were cultivated, and in time, the researchers from V.R.D.S. Buzău achieved valuable genotypes that were very appreciated both by growers and consumers, among which the variety 'Dragaica', destined for protected areas and open field cultivation, the 'Zaraza' variety destined only for open field cultivation and, recently the F1 hybrid 'Rebeca' has been obtained, with a mixed destination, protected areas and open field culture.

Researches were constituted among achieving a rich germplasm collection for this species, comprising local populations, accessions, autochthonous and foreign varieties. "Most species within *Solanum* are endemic to the Americas; however, ~20% is Old World species. The common name eggplant encompasses three closely related cultivated species that belong to *Solanum* subgenus Leptostemonum: *Solanum melongena* L., brinjal eggplant or aubergine; *S. aethiopicum* L., scarlet eggplant; *S. macrocarpon* L., gboma eggplant." (Daunay et al., 2001).

Among the old and traditional cultivars, an emphasis was put on preserving this species biodiversity.

"The first center of diversity for eggplants is in India, and the second one in China." (Ramalho do Rêgo, 2012)

Thorough specific breeding work programs we can achieve new distinct genotypes.

"Eggplants (*Solanum melongena* L.) were domesticated in tropical Asia where they are used abundantly as both food and medicine. Human selection has produced hundreds of landraces that differ in morphology and chemistry in ways that may be related to local ethnobotanical preferences." (Meyer et al., 2012).

MATERIALS AND METHODS

Researches were targeted on collecting valuable genetic material and structuring it based on the breeding objectives. The general

field collection has a large number of genotypes that are in different stages of breeding, among which 84 accessions were promoted in the working field and submitted to an intensive breeding program. Among these, 36 accessions are genetically stabilized, 23 are in an advanced stage and 25 are still segregating. From all the 36 stabilized accessions, 11 part of this paper due to their distinct phenotypic expressivity.
The breeding methods used were repeated individual selection, hybridization, segregation and negative mass selection. The crop technology used both for protected areas and open field was the species specific one. Crop establishment was made through seedlings aged of 55-60 days.
For the open field crop, the planting scheme used was of 70 cm between rows and 35-40 cm between plants on rows, and for the protected areas, the crop establishment was made in bands at 70 cm between rows, 1.2 m between bands and 40 cm between plants on the rows (Figures 1 and 2).

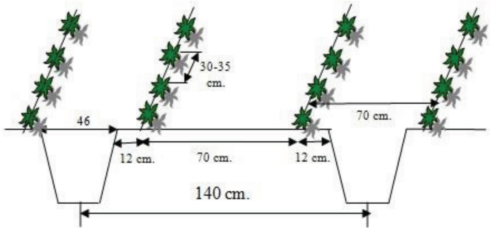


Figure1. Planting scheme for protected areas

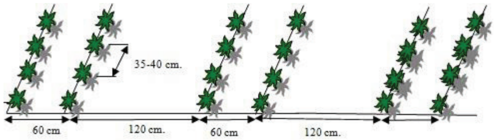


Figure2. Planting scheme for open field

RESULTS AND DISCUSSIONS

The research conducted since 1990 at V.R.D.S Buzău, finalized with the achievement of ten genetically stabilized accessions and with distinct phenotypic expressivity: A 1 S, A 7, A 10, A 20, A 25 A, A 22 A, A 23 A, A 24 A, A 29 A, A 29 B.
The main plant characteristics are presented in table 1.
The values recorded in table 1 demonstrates visible distictibility between the accessions in what regards the main traits of the eggplants. Regarding the plant height, on the first place is A 29 B, with a medium height of 180 cm and the lowest value was recorded at L 20 with 112 cm height. Differences were recorded regarding thr anthocianic coloration. It was founded that the accessions A 1 S, A 22 A and A 23 A have a strong anthocianic pigmentation on the stem, sprouts and on the main leaf veins. Due to this characteristic these eggplants weren’t preffered by the Colorado beetle.
The foliage is an important marker for distictivness for all the new creations. Important differences were observed regarding the length of the stalk, the length and width of the lamina, and leaf type.

Table 1. The main characteristics of the plants, mean values

Character/accession	Rebeca F1	L1 S	L7	L 10	L 20	L 25 A	L 22 A	L 23 A	L 24 A	L 29 A	L 29 B
Plant height(cm)	155	147	125	120	112	129	128	118	126	156	180
Stem height (cm)	14	26	13	9	13	22	33	19	47	8	19
Main sprouts no.	3	4	2	5	2	2	2	4	4	3	4
Main sprouts length (cm)	116	123	73	91	99	83	87	98	71	115	103
Stem diameter (cm)	2.2	2.3	1.5	2.3	1,6	1.4	1.9	1.7	1.5	2.1	1.8
Peduncle length (cm)	13	11	11	11	11.5	15	8	8.5	11	10.5	12
Lamina length (cm)	27	21	14.5	29	25	35	29.5	30.5	33	33.5	31
Lamina width (cm)	19	18	17.5	22	15.5	20	20	18.5	15.5	22	27
Flower color	Mauve	Mauve	Purple	Purple white	Mauve	Purple white	Purple	Mauve	Mauve	White	White
Corolla diameter (mm)	35	32	48	45	31	42	35	40	53	24	20
Anthocianic pigmentation	-	Stem, sprouts, leaf, sepals	-	-	-	-	Sprouts, main and sec. veins	Stem, main veins	-	-	-

A special attention for fruit setting was given in the breeding. The accessions achieved are distinguished by productivity, quality, earliness and genetic resistance to the main specific diseases. The researches conducted had as the main purpose, the assortment enrichment with new cultivars at this species, valuing its genetic potential by introducing new totally different genotypes beside the old, classic ones.
A special emphasis, was on the color and shape of the fruit, correlated with the directions of use (Figure 3.).

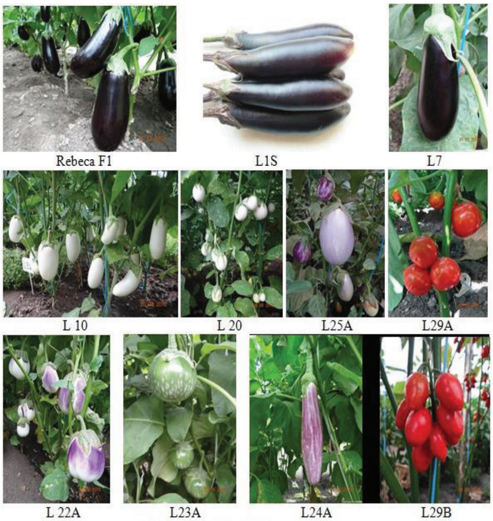


Figure 3. Main accessions studied

The fruit main traits are presented in table 2. Throughout the experience, V.R.D.S. Buzău managed to obtain the F1 hybrid Rebeca, a hybrid that can be used in protected areas, but also in a open field crop.
It is presented as a globular bush, with an average height of 155 cm and has no spines. The color of the fruit is black and shiny, cilindrically shaped.
It preserves the characteristics of the traditional eggplants that have a large fruit. The average fruit weight is of 590 g, has a small number of seeds that are mostly present in the apex of the fruit. This variety is genetically resistent at the main pathogens. Average production per plant is registered with 3.5 kg.
A 1 S has long black fruits, sword-type, with an average length of 26 cm and 8.4 cm median diameter. This accession is distinguished by a high productivity, strength, the presence of anthocianic pigmentation on the plants vegetative organs, including the fruit sepals. Due to it's strength and strong radicular system, well developed, it can be recomended to be used as a rootstock. This genotype has the "*mi*" gene, known as a repelent gene for nematodes. The average plant production is of 2.8 kg.
A 7 has medium length fruits, with green sepals, a small number of seeds dispersed throughout the length of the fruit. The average fruit weight is of 457.4 g, and 2.7 kg fruit/plant production.
A 10 has large white fruits, a trait that distinguishes it from the other varieties, along with a reduced number of seeds. It is highly productive, with fruits that have a good quality, and the average production per plant is of 4.1 kg. Due to it's exceptional traits, productivity, earliness, along with fruit quality and good process capacity made this genotype to be highly demanded by growers and consumers, and the request for seeds and seedlings grew significantly year by year.
A 20 is a premier for our country, especially for culinar dishes; it is distinguished by its small white, egg-shaped fruits. It can be used in a great variety of dishes in order to replace mushrooms, or can be pickeled as cucumbers and mushrooms. It has a large variety of fruit uses and it can also be used as in an ornamental system. The fruit has an average weight of 49.2 g and the registred production/plant is of 1.7 kg.
A 25 A is distinguished by white fruits that have purple shadows, and a mean fruit weight of 155.5 g with an average production/plant of 1.3 kg. Regarding the cpacity of production is below the other genotypes, but, due to its shape and colour it is highly demanded both by consumers and growers.
Another trait of distinctibility are the spines that are very much present on the sepals and peduncle, giving it a rustic aproach. Also, it was recorded an increased resistance to specific diseases, especially to *Verticilium*.
A 22 A has large, obovate, white with purple patches fruits. They are over 600 g, and as a special trait has ribbs. This cultivar is remarkable by its productivity, obtaining the highest production/plant, 4.7 kg. All of this features made this genotype to have a great

request from growers and consumers, for open field crops and for protected areas.

A 23 A, a cultivar remarked by the shape and colour of the fruits, white with green patches, small rounded shape.

The average weight is 101.6 g and the mean production/plant is of 1.3 kg. Its shape and colour makes it very special, and we believe it will have great opportunities for the future.

A 24 A is distinguished by its productivity, quality, and the main feature is represented by the fruit colour. It has stripped fruits, white with mauve stripes. The fruit average weight is 530 g and the total production per plant is of 3.7 kg. Due to its attractive shape and colour that gives it a special appearence, this genotype is more and more demanded by the market.

Table 2. Fruit characteristics

Character/accession		Rebeca F1	L1 S	L7	L 10	L 20	L 25 A	L 22 A	L 23 A	L 24 A	L 29 A	L 29 B
Peduncle length (cm)		12	5.9	7.3	9,8	2.9	6.6	5	5.6	6.3	1.8	1,6
Spines presence on		Sepals	-	Sepals	Peduncle	Sepals	Sepals, peduncle	Sepals	-	Peduncle, sepals	-	-
Sepals colour		Green	Green	Green	Light green	Light green	Green	Green	Light green	Light green	Dark green	Green
Fruit length (cm)		24.3	26	18.5	18	5.4	12.6	10.5	5.2	14	5.5	4.3
Fruit diameter (cm)	Apex	6	4.3	7,1	7.5	3.6	6.1	10.7	5.2	7.9	5.7	1.6
	Median	8.4	5.7	6,4	9.1	4.4	8.5	12.5	6.3	9.6	6.3	2.4
	Base	5.4	4.4	4,5	7	4.3	6.9	10.7	5.2	7.2	5.7	1.8
Fruit colour	Technological maturity	Black	Black	Black-mauve	White	White	White + mauve	White + mauve (patches)	White/ green patch	White+ mauve patches	Stripped green	Beige+ green
	Physiological maturity	Black	Black	Dark mauve-dark green	Yellow	Yellow	Yellow+ mauve	Yellow with mauve patches	Yellow	Yellow+ mauve	Red with green stripes	Dark orange
No of. fruits/plant		6	7	6	7	36	9	7	13	7	16	81
Fruit weight (g)		590	412.6	457.4	592	49.2	155.5	685	101.6	530	106.6	11.4
Total fruit weight/pl. (kg)		3.5	2.8	2.7	4.1	1.7	1.3	4.7	1.3	3.7	1.6	0.92

A 29 A is a new valuable genotype obtained at V.R.D.S. Buzău, that is distinguished by green fruits that colours in red at the physiological maturity, with small flat-globular fruits. The fruit has an average weight of 106.6 g and a total production/plant of 1.6 kg. This cultivar is characterized by a great vigour, making it suitable for the ongoing tests as a rootstock. So far, the preliminary results showed a great compatibility between it and the scions used, imprinting them a great resistance to the specific pests and pathogens.

A 29 B is a special variety that is distinguished by its pepper-type flower, and and linear raceme type inflorescence. The fruits are small, ovoid, beige with small green stripes. The average fruit weight is of 11.4 g and total production/plant is 0.9 kg. This cultivar is also being tested as a rootstock, with very good results.

CONCLUSIONS

Researches finalized by setting a solid germplasm collection, evaluation and computerized data gathering of new stable in lineage genotypes. From all of them, due to its special appearance and features, A 10 was in 2017 registered at ISTIS Bucharest to be patented under provisional name ‘Camelia’.

The achieved genetic resources allow us to obtain new valuable cultivars.

REFERENCES

Doganlar S., Frary A, Daunay MC, Lester RN, Tanksley SD., 2002. A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae, Genetics 161(4): 1697-1711.

Meyer R.S., 2012. Chemical, genetic, and ethnobotanical diversity in Asian eggplant. City University of New York.

Ramalho do Rêgo E., Nascimento M.F., do Nascimento N.F.F., Fortunato F.L.G., Finger F.L., Monteiro do Rêgo M., 2013. Heterosis for fruit quality traits in ornamental peppers, Breakthroughs in the Genetics and Breeding of Capsicum and Eggplant, Torino, 2-4.09.2013; 701

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CURRENT STRATEGIES FOR THE PROTECTION OF ORGANIC CROPS IN VEGETABLES PRODUCTION

Steliana RODINO[1], Marian BUTU[1], Gina FIDLER[1], Ancuța MARIN[2], Alina BUTU[1]

[1]National Institute of Research and Development for Biological Sciences, Splaiul Independentei, no 296, P.O. Box no 17-16, 060031, Bucharest, Romania, Tel. / Fax. +40212200880
[2]Research Institute for Agricultural Economics and Rural Development, 61 Blvd. Marasti, 011464, Bucharest, Romania

Corresponding author email: marian.butu@yahoo.com

Abstract

Development of disease resistance to conventional pesticides and environmental contamination problems created pressure on farmers to adopt new strategies for disease control in the production of vegetables. In addition, consumers demand to minimize pesticide residues in food products is forcing the growers and the pesticide producing companies to formulate and develop alternatives to the contentious inputs currently marketed. Products obtained from biologically active compounds extracted from plants will play an increasingly important role in crop protection strategies. Exploiting antimicrobial substances from plants that inhibit or halt the reproduction of pathogenic microbes, would become a more realistic and ecological method for the integrated management of plant diseases with the final goal of reducing or gradually phasing out contentious inputs without compromising the competitiveness of the organic sector. Obtaining, identifying, testing and physicochemical characterization of biologically active compounds with action to combat microbial diseases in vegetable crops shows both originality and complexity of activities proposed in the research work. The present study provides an overview of the current state of external inputs use and proposes a solution to the stricter European standards, by a systemic approach of biotechnological sciences and agricultural sciences, with immediate applicability of the obtained results in farm practices.

Key words*: organic systems, vegetables, natural products, crop protection, plant products.*

INTRODUCTION

The continuous growing of consumers demand for organic food lead to the need to develop solutions for increasing both production and quality obtained from organic systems.

Organic farming is a cultivation method that does not use pesticides or chemical fertilizers, and that replaces them with other methods including products obtained from plants or animals: purine, compost, mulch.

Organic production is defined as a holistic system that integrates cultural, biological, and mechanical practices that foster cycling of natural resources adapted to local conditions, promote ecological balance by avoiding external inputs with high resilience, and conserve biodiversity (IFOAM, 2009, Regulations of department of Agriculture US, 2011).

Development of pest and disease resistance to conventional pesticides and environmental contamination problems created pressure on farmers to adopt new strategies for disease control in horticultural production, both for conventional and organic systems.

It is expected that products obtained from biologically active compounds extracted from plants will play an increasingly important role in future crop protection strategies. The exploitation of antimicrobial substances from plants that inhibit or halt the reproduction of pathogenic microorganisms, would become a more realistic and ecological method for the integrated management of plant diseases and pests with the final goal of reducing or gradually phasing out contentious inputs, without compromising the competitiveness of the organic sector.

The present study provides an overview of the current state of external inputs use and proposes a solution to the stricter European standards, by a systemic approach of biotechnological sciences and agricultural sciences, with immediate applicability of the obtained results in farm practices. The high degree of originality and innovation in organic research lies in testing by detailed analysis of the designed solutions and its conceptual framing in standards provided by Directive 91/414/CEE, 15 July 1991 for Plant Protection Product. This study puts together, in an original way, both material resources from the field of vegetable crops and from technology aimed to decrease the microbial diseases attack.

SUSTAINABLE STRATEGIES USED IN PLANT PROTECTION MANAGEMENT

Biological control

Biological control is defined as the use of living organisms to control pests or the use of microbial antagonists to suppress diseases. There is a growing demand for biologically based pest management practices. Recent surveys of both conventional and organic growers indicate an interest in using biocontrol products suggesting that the market potential of biocontrol products will increase in the future (Heydari et al., 2010).

Biocontrol agents may be predatory, parasitic, or pathogenic; they may also be either "natural" (from naturally occurring organisms in the ecosystem such as wild beneficial insects) or "applied" (meaning external organisms and microorganisms that are introduced in the agrosystem). Biocontrol agents include insects, mites, bacteria, fungi, viruses, and nematodes.

In the recent years, extensive research is being carried on beneficial bacterial isolates that proved to have antimicrobial effects against various phytopathogens.

In vivo tests pointed out *Paecilomyces variotii* as a broad spectrum biocontrol agent effective against both bacterial spot of tomato and *Fusarium* wilt of melon (*F. oxysporum* f.sp. *melonis* and *X. campestris*) (Suarez et al., 2013).

Several fungal and bacterial biocontrol agents have been used as seed and soil application to reduce the incidence of plant diseases caused by soil borne fungal pathogens (Heydari et al., 2010).

Plant extracts

Traditionally, the plant extracts were used in the form of plant extracts and volatile oils. Aromatic and medicinal plants have attracted interests in the field of plant disease control, particularly plant extracts with antimicrobial properties and contain a spectrum of secondary metabolites such as alkaloids, quinolones, flavonoids, glycosides, saponins, tannins and terpenoids (Sales et al., 2016). Reports on medicinal plants extracts have shown *in vitro* inhibitory effects against phytopathogens (Rodino, 2013; Harlapur et al., 2014; Prashith K.T.R. et al., 2010). Plant extracts have also been reported to have antimicrobial properties against plant pathogens including viruses, fungi, bacteria, nematodes and insects *in vitro* as well as *in vivo* (Duraisamy, 2015).

Allelopathy

The term of allelopathy was first used in 1937 by Molisch, defining the chemical interaction (both stimulation and inhibition) between all types of plants, including microorganisms (Kalinova J., 2010; Bostan et al., 2013). Allelopathic plants are basically used as catch crops or trap crops. They are used in plant protection of tropical regions against parasite weeds, reducing the parasite seed bank by 72%. Allelopathic compounds can act as insects repellent (Kalinova J., 2010). Good results were also obtained for parasite weed control.

Intercropping

Intercropping is the planned mixed growing of more than two species at one area of land in the same period of time. A prerequisite for the success of intercropping is the interdependence of the selected crops in growth and development due to their biological particularities. The method is based on complex interactions between companion species with good results in trap cropping, weed suppression, physical-spatial interactions.

Intercropping effects are not only reducing pest populations, it proved to be efficient. in controlling plant diseases. The grain intercrop reduced humidity in the canopy and reduced the raindrop splash effect, those two conditions being the favorable to fungal spores spread (Schoeny et al., 2008). Moreover, studies showed that intercropping system is more productive than single cultivation system due to complementing effects of the companion crops.

CURENT CHALLENGES

The complexity of organic farming principles requires farmers to achieve a high level of knowledge and skills. The resources available in organic agriculture are presently limited due to very strict rules in force. Basically, crop protection solutions are limited to Integrated Pest Management solutions and the use of crop protection chemicals is drastically fenced, being allowed only few basic substances.

Basic substances allowed in organic agriculture are set by EU commission, and they consist of materials that are covered by the definition of food stuff Regulation (EC) No 178/2002 and have plant or animal origin. Basic substance is defined as active substance simultaneously fulfilling the criteria:

a) is not a substance of concern;

b) does not have an inherent capacity to cause endocrine disrupting, neurotoxic or immunotoxic effects;

c) is not predominantly used for plant protection purposes but is useful in plant protection either directly or in a product consisting of the substance and a simple diluent;

d) is not placed on the market as a plant protection product (EC, 2009).

Products based on natural components used against phytopathogens gained attention in search of environmentally friendly solutions usable either for mass production or for organic and low-input farming systems. After validation of antimicrobial effects of medicinal plants used in traditional medicine, the research aim expanded to evaluation of plant based products against phytopathogens (Rodino et al, 2013). Research on medicinal plants extracts and oils showed the inhibitory effects against various phytopathogenic fungi

In vitro studies on some species of plants such as weeds or trees with less known medicinal value, that could be used to control plant diseases and pests were also reported (Srivastava et al., 2011; Mahlo et al., 2010).

It was demonstrated the antifungal bio-capacity on powdery mildew, downy mildew and botrytis of grape cane extracts in greenhouse and field assays (Houille B. et al., 2015). These extracts were obtained after a long alcoholic extraction by hot soxhlet using large amounts of solvents. A recent study (Soural I. et al., 2015) describes best extraction techniques, but the results show importance of methanol, a toxic laboratory solvent not usable in green technology and prohibited in the homologation category as basic substances.

FUTURE TRENDS

Scientific knowledge on the preparation mode of plant based products and how to use them are rather rare and incomplete. Therefore, it is important to optimize the extraction and manufacturing process, the stabilization of these preparations, dates and rates of application.The support of the use of these natural preparations or substances, as well as their market authorisation, requires their evaluation and registration within Plant protection Products Register. Plant protection product data must be collected from research on alternative or traditional crop protection methods (Marchand, 2015).

Another promising hypothesis for organic farming is the use of bacterial preparations based on nitrogen-fixing microorganisms. They are introduced into the root zone of plants, thus enriching the soil with biological nitrogen.

CONCLUSIONS

Development of pest and disease resistance to conventional pesticides and environmental contamination problems created pressure on farmers to adopt new strategies for disease control in horticultural production, both for conventional and organic systems.

Obtaining, identifying, testing and physico-chemical characterization of biologically active compounds with action to combat microbial diseases in vegetable crops implies both originality and complexity of activities proposed in the research work.

Moreover, plant protection product data must be collected from research on alternative or traditional crop protection methods.

Preliminary trials concerning antimicrobial potential of plant extracts are encouraging but full characterization and optimization is still required. However, to be fully efficient at farm level, proof of concept functionality has to be tested, and technical data sheets have to be produced.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2016-1544, contract number 14PED/2017, within PNCDI III.

REFERENCES

Bostan C., Butnariu M., Butu M., Ortan A., Butu A., Rodino S., Parvu C., 2013. Allelopathic effect of *Festuca rubra* on perennial grasses, Rom. Biotech. Letters, 18(2), 8190-8196.

Duraisamy S.,Loganathan K.,Rajendran R.,Kuppusami P.,Thiruvengadam R., 2015. Characterization of BioactiveCompounds from Botanicals for theManagement of Plant Diseases In Book Sustainable Crop Disease Management using Natural Products, Cabi.

EC (2009) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009, Official Journal of the European Union L309, 1–50.

Harlapur S.I., Kulkarni M.S., Wali M.C., Srikantkulkarni H., 2007. Evaluation of Plant Extracts, Bio-agents and Fungicides against Exserohilum turcicum (Pass.) Leonard and Suggs. Causing Turcicum Leaf Blight of Maize, India, J. Agr. Sci.20(3), 541-544.

Heydari A., Pessarakli M., 2010. A Review on Biological Control of Fungal Plant Pathogens Using Microbial Antagonists. Journal of Biological Sciences, 10: 273-290.

Houille B., et al., 2015, Biosynthetic origin of E-resveratrol accumulation in grape canes during postharvest storage. Journal of Agricultural and Food Chemistry 63, 1631-1638.

International Federation of Organic Agriculture Movements (IFOAM 2009).

Kalinova J., 2010, Allelopathy and Organic Farming, Sociology, Organic Farming, Climate Change and Soil Science Sustainable Agriculture Reviews, 3, 379-418.

Mahlo S.M., McGaw L.J., Eloff J.N., 2010. Antifungal activity of leaf extracts from South African trees against plant pathogens, Crop Protection 29, 1529-1533.

Marchand P.A, 2015. Basic substances: an opportunity for approval of low-concern substances under EU pesticide regulation Pest Management Science, 71 (9), 1197–1200

Prashith K.T.R. et al., 2010. Screening of selected single and polyherbal Ayurvedic medicines for Antibacterial and Antifungal activity, Anc Sci Life, 29(3),22–25.

Regulations of the Department of Agriculture, 2011. Agricultural Marketing Service (Standards, Inspections, Marketing Practices), Organic Foods Production Act Provisions, 7 CFR 205.2 - Terms defined.

Rodino S, et al., 2013. Investigation of the antimicrobial activity of extracts from indigenous *X. strumarium* plants against *P.infestans*, Curr Opin Biotech, 24, S72-S73.

Sales, M.D.C.; Costa, H.B.; Fernandes, P.M.B.; Ventura, J.A.; Meira, D.D., 2016. Antifungal activity of plant extract with potential to control plant pathogens in pineapple. Asian Pacific J. Trop. Biomed., 6, 26-31.

Schoeny A., Menat J., Darsonval A., Rouault F., Jumel S., Tivoli B., 2008. Effect of pea canopy architecture on splash ispersal of *Mycosphaerella pinodes* conidia. Plant Pathology. Vol. 57., 1073–1085.

Soural I., et al., 2015. Various Extraction Methods for Obtaining Stilbenes from Grape Cane of *Vitis vinifera* L. Molecules, 20, 6093-6112.

Srivastava D., Singh P., 2011. Antifungal Potential of Two Common Weeds against Plant Pathogenic Fungi, Asian J. of Biological Sciences, Volume 2(3), 525-528.

Suárez-Estrella F., Arcos-Nievas M.A.,. López M.J, Vargas-García M.C., Moreno J., 2013. Biological control of plant pathogens by microorganisms isolated from agro-industrial composts, Biological Control, 67, 3, 509–515

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON CONSERVATION, EVALUATION AND GENETIC HERITAGE EXPLOITATION OF TOMATO

Bianca ZAMFIR[1], Dorel HOZA[2], Costel VÎNĂTORU[1], Viorica LAGUNOVSCHI[2], Camelia BRATU[1], Elena BĂRCANU[1]

[1]Vegetable Research and Development Station Buzău, No. 23, Mesteacănului Street, zip code 120024, Buzău, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: zamfir_b@yahoo.com

Abstract *Concerns on tomato breeding at V.R.D.S. Buzău existed since the institution settlement, in 1957. Over time, Breeding Laboratory created valuable tomato varieties with a well-defined genetic constitution, appreciated by growers and consumers. Many old varieties, created between 1970-1980 (ex. 'Buzău 1600', 'Buzău 22', 'Buzău 47') were kept genetically pure being on high demand nowadays. After 1996, the Breeding Laboratory restarted intensive research on tomato breeding. Particular attention was paid to purchase genotypes, accessions, varieties, local populations and foreign genotypes with the aim of forming a solid germplasm collection. Secondly, the germplasm collection that is steadily growing, currently reaching over 1500 genotypes, was evaluated, distributed on two fields, collection field and work field. Particular attention regarding the assessment was given to the genetic stability and type of plant growth. In terms of genetic stability, cultivars evaluated were grouped as: stable, advanced and segregating. Concerning the type of growth SP gene expressivity was followed and genotypes were grouped as follows: genotypes with indeterminate growth (SP +) semi determinate genotypes (Sp) and determinate genotypes (sp). Up to now, breeding works were completed with the approval of new genotypes and their widespread as cultivated crop. Among the recently approved cultivars are: 'Siriana' F1, 'Kristinica', 'Darsirius', 'Chihlimbar', 'Florina', 'Ema de Buzău', and for pending approval 'Măriuca', A 80 ('Estera'), A 2000 ('Bizon'), A 28 ('Hera'). Genetically stable accessions were tested through general and specific combining ability in order to develop new hybrids.*

Key words: *breeding, biodiversity, hybridization.*

INTRODUCTION

Among the vegetables grown in Romania, tomato is the most cultivated species both as crop and for fresh market.

Due to its Latin American origin and related domestication history, cultivated tomato has faced several bottlenecks over ages. This led to a drastic reduction of its genetic diversity. Explorations of tomato center of origin permitted major advances in the characterrization of its diversity (Bauchet, 2012).

Concerns on tomato breeding at V.R.D.S. Buzău existed since the institution settlement, in 1957.

At the beginning were cultivated local populations and foreign cultivars, especially Bulgarian.

As a result, its genetic basis has been seriously narrowed, known as the 'domestication syndrome' (Aflitos, 2014)

For the first time in Romania, at Vegetable Research Development Station Buzău hybrid seed was obtained by known commercial tomato Bulgarian hybrid 10X Bizon.

Later, here were obtained the first Romanian tomato varieties as follows: 'Buzău 1600', 'Buzău 22', 'Buzău 47', 'Diana'.

Traditionally, tomato improvement has been carried out by classical breeding approaches by introgression genes from the wild relatives (Mohan, 2016).

V.R.D.S. varieties have well-defined genetic constitution and showed durability unaltered for over 40 years. The varieties are required and appreciated by growers and consumers even today.

MATERIALS AND METHODS

After 1996, research on tomato breeding at V.R.D.S. Buzău resumed intensively after a well-organized schedule:

Phase I objective was a continuous enrichment and improvement of germplasm collection in this species.

Phase II aimed to evaluate the germplasm collection and its distribution on fields and breeding phases.
Phase III aimed the introduction of valuable genotypes in intensive breeding works.
Phase IV aimed to develop germplasm collection by acquiring new genotypes, varieties and hybrids.
Germplasm collection has been divided into distinct groups according to the type of plant growth and genetic stability (Figure 1).

Figure 1. Germplasm collection composition and breeding phases:

SP+ (indeterminate accessions) - 887 accessions from which S (Stable) 326, A (advanced)- 253 and Sg (segregating)-308;
SP (semi determinate accessions) - 296 accessions from which S (Stable) 91, A (advanced) – 78 and Sg (segregating)-127;
Sp -(determinate accessions)- 341 accessions from which S (Stable) 112; A (advanced)- 98 and S (segregating)-131

Collected germplasm resources were evaluated in terms of stability and their use in breeding process according to the following plan (Figure 2):

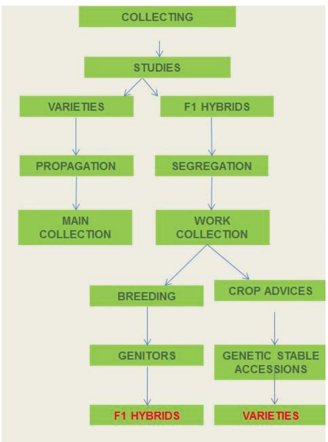


Figure 2. The use of germplasm resources of tomato

The breeding process emphasised the use of genotypes with distinct phenotypic expression. Among them stood out particular accessions as: A 524, A 203, A 532, A 208, A 609 (Figure 3).

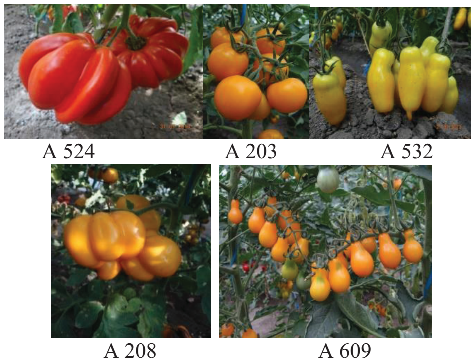


Figure 3. Genotypes with distinct phenotypic expression

Besides the main goals of breeding: productivity, earliness, crop quality, was followed the obtaining of cultivars with genetic resistance to species pathogens (Figure 4).

Figure 4. Wild tomato containing valuable gene for breeding process

In breeding process were used genitors with the nematode resistance *mi* gene and genitors with vigourous and healthy root system selected and recommended as valuable parent stocks (Figure 5).

Figure 5. Wild tomato, recommended as parent stocks

RESULTS AND DISCUSSIONS

The genetic stable genotypes were tested in the process of general and specific combining ability.

For general and specific combining ability test were used as tester genitors the following accessions: SP+, A 19, A 24 x13, A 165, A 23 and self-pruning accessions A 10, A 22, A 46, A 50, A 66 and A 67.

Results obtained from general combining ability test of the 326 genetic stable genotypes with indeterminate growth (+ SP), a total of 182 have passed the general combining ability.

Of the 91 genetic stable genotypes with semi-determinate growth (SP), a total of 38 have passed the general combining ability.

Of the 112 genetic stable genotypes determinate (sp), a total of 57 have passed the general combining ability.

Results obtained from specific combining ability test of the 66,248 possible hybrid combinations for SP+ genotypes, for testing specific combining ability, there were made a total of 642 hybrid combinations.

Of the 2,888 possible hybrid combinations for SP genotypes, in order to achieve specific combining ability, it has been made a number of 86 hybrid combinations.

Of the 6,498 possible combinations hybrid for sp genotypes to achieve specific combination, it was performed a total of 184 hybrid combinations.

Valuable hybrid combinations obtained by testing specific and general combining ability (Figure 6).

30 hybrid combinations showed visible reproductive, somatic, metabolic, adaptive heterosis phenomenon.

To prove the stability and uniformity of the parents and the compatibility between them and the uniformity of the hybridization results, hybridization process was repeated for six years so far.

As hybrid control variant in the experiments was used 'Siriana' F1 hybrid (Figure 7).

Valuable lines that demonstrated lineage genetic stability and correspond to breeding objectives were approved and patented and registered in the Official Catalogue of Crop Plants of Romania under the following names: 'Ema de Buzău', 'Chihlimbar', 'Kristinica', 'Darsirius', 'Florina', 'Măriuca'.

H. F1	Yield/PL. (KG)	H. F1	Yield/PL. (KG)	H. F1	Yield/PL. (KG)
H1BZ	4,200	H9S	4,200	H20	6,750
H1SA	3,000	H10S	3,795	H21	4,100
H1SB	3,300	H11S	5,270	H22	4,350
H2S	4,500	H12S	5,340	H23	6,700
H3S	4,560	H13SA	6,150	H24	4,150
H4S	4,396	H13SB	6,100	H25	4,350
H5S	5,800	H14SA	6,100	H26	6,850
H6S	3,000	H14SB	6,200	H27	3,850
H7S	4,752	H15SA	6,600	H28	4,600
H8S	3,654	H15SB	6,500	H29	3,950
				H30	4,194

Figure 6. Hybrid combinations that expressed heterosis phenomenon

Figure 7. Hybrids (H15, H14, H11 and H12)

In addition, there were a total of three lines pending approval with the following proposed names: L 2000 ('Bizon'), L 80 ('Estera') and L 28 ('Hera').

Varieties suitable for industrialization were biochemically analyzed, placing particular emphasis on the dry matter content, acidity, sugar content of the fruits etc.

Tomato varieties suitable for fresh market

'EMA DE BUZĂU', indeterminate variety, suitable for both greenhouse and field. The plant can reach a height of 2.5-3 m in greenhouse and 1.8-2.2 m in field.
It has an high yield/ plant, 3 kg in greenhouse and 2-2.5 kg as field crop.
It presents a high number of fruits per plant of over 280 red colored and tiny, 6-10 g/fruit with medium consistency.
Strong character of this variety is given by the unique taste and flavor.

'CHIHLIMBAR', determinate variety, suitable as crop field. The cultivar presents a vigorous plant, with an average height of 85 cm, big fruits, orange colored. The fruit weights 225 g, yield/plant-2.5 kg and the fruits can be harvested without peduncle.

Tomato varieties suitable for industrialization

'KRISTINICA', determinate variety, with an average height of 60 cm, small vigour and reduced foliage. The variety presents an average number of 6 fruits/ truss. Before reaching maturity the fruit has green shoulders (*U* gene).
The fruit is round shaped and weights 120 g. the fruits are consistent and red colored.
Yield/plant-2.5 kg and 50 – 60 t/ha.

'DARSIRIUS', determinate variety, semiearly, suitable for field and industrialization, the plants height is between 50-60 cm with 4-6 vigorous shoots. The fruits are ovoid shaped with an average weight above 80 g. Can be easily harvested without peduncle.

'FLORINA', determinate variety, suitable as field crop. The fruits has an average weight of 160 g, round shaped, slightly ribbed, suitable both for fresh market and processing. Yield/plant- over 2.5 kg.
'MĂRIUCA', determinate variety, suitable as field crop and processing with big ovoidal fruits.

The industry varieties were biochemical analysed and it was found that in the case of dry matter content, due to the pericarp density, 'Darsirius' variety ranked the first place with 5.8%; dry soluble matter remains at the rate of 5% as in the case of the variety 'Kristinica' In the case of varieties of 'Florina' T, R and r. Daria ('Măriuca') an equal value of 4.5% was recorded. Also, 'Kristinica' has registered a value of 0.43% acidity, at the opposite side being 'Darsirius' with 0.35%. The highest content in sugar was recorded by 'Darsirius' while Daria ('Măriuca') has registered a rate of 2.44 percent (Figure 8).
Regarding the sugar: acidity ratio, 'Darsirius' was first valued with 8.97%, followed by 'Florina' R with 16%. The highest content in ascorbic acid was measured at 'Florina' T followed by 11.97% at 'Darsirius' with 9.58%. The highest content of lycopene 9.08% was recorded by 'Florina' R (Table 1).

Tomato varieties pending approval

Accession 80 ('**Estera**'), indeterminate variety, suitable for both fresh market and industrialization, ovoid fruits which weight 16-20 g with a reduced number of seeds, less than 20 seeds/fruit.

Accession 2000 ('**Bizon**'), indeterminate variety, suitable as crop field trained to a trellis. The genitors originate from a heirloom tomato called "Ox heart". It presents very large fruits ranging from 300-950 g, heart-shaped, with green cap which disappears when reaching physiological maturity.

Accession 28 ('**Hera**'), indeterminate variety with red fruits long pepper shaped. The water excess causes fruit cracks on the shoulders.
The variety has a high yield/ plant, suitable for fresh market (Figure 9)

Table 1. The biochemical analysis of tomato fruits for industrialization

Parameter	Variety					
	'Măriuca'	'Kristinica'	'Florina' T	'Florina' R	'Darsirius'	Average values*
d.m. %	5.18	5.26	5.35	5.30	5.8	5.98±0.83 (cv=14%)
s.s. %	4.5	5.00	4.5	4.50	5.00	4.75±0.35 (cv=7%)
Acidity (as Citric acid),%	0.41	0.43	0.39	0.37	0.35	0.38±0.03 (cv=8%)
Sugar,%	2.44	2.97	2.88	3.02	3.14	3.37±0.77 (cv=21%)
Sugar acidity level	5.95	6.91	7.38	8.16	8.97	10.76±0.09 (cv=1%)
Ascorbic acid, mg/100 g^{-1}	8	7.02	11.97	8.30	9.58	9.67±0.65 (cv=7%)
Lycopene, mg/100 g^{-1}	6.5	5.00	8.18	9.08	6.00	8.00±1.5 (cv=19%)
Average weight, g± a.s.	93.5±22.1 (cv=24%)	108.9±13.5 (cv=12%)	139.9±29.1 (cv=21%)	167.9±26.0 (cv=15%)	86.9±13.3 (cv=15%)	92±10 (cv=11%)

** Viorica and Vipon varieties (ICDLF Vidra); d.m.-dry matter content; s.s.-soluble solids; Gr.-weight*

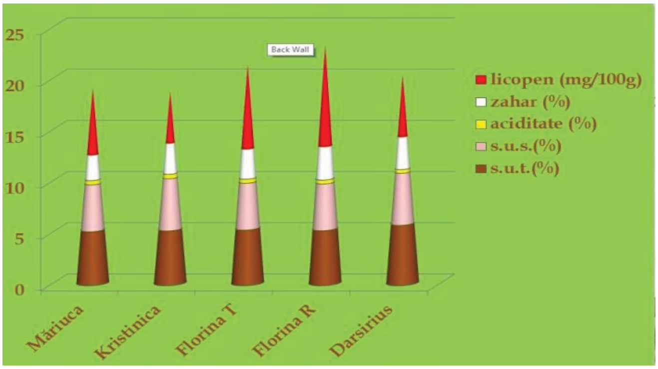


Figure 8. The biochemical analysis of tomato fruits for industrialization


'Ema de Buzău'


'Estera'


'Kristinica'


'Darsirius'


'Florina'


'Măriuca'


'Chihlimbar'


'Bizon'

Figure 9. Tomato varieties

CONCLUSIONS

The researches have been completed so far with making a valuable germplasm collection consisting of 1524 genotypes;

Evaluation and division of germplasm collection on collection fields and work fields;

Genetic stable genotypes were evaluated in terms of general and specific combining ability;

It was obtained a total of 30 valuable hybrid combinations which expressed visible heterosis phenomenon;

It was patented a hybrid and six varieties and three varieties are undergoing approval.

REFERENCES

Aflitos S. et al., 2014. Exploring genetic variation in the tomato (Solanum section Lycopersicon) clade by whole-genome sequencing, Plant Journal 80(1): 136-148.

Bauchet G., Causse M., 2012. Genetic diversity in tomato (Solanum lycopersicum) and its wild relatives, 133.

Mohan Vijee et al., 2016. Tomato fruits show wide phenomic diversity but fruit developmental genes show low genomic diversity, PlOS one 11(4): e0152907.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CHARACTERIZATION OF NEW ORNAMENTAL CHILLI GENOTYPES CREATED AT V.R.D.S. BUZĂU

Elena BĂRCANU[1], Costel VÎNĂTORU[1], Bianca ZAMFIR[1], Camelia BRATU[1], Elena DRĂGHICI[2]

[1]Vegetable Research and Development Station Buzău, No. 23, Mesteacănului Street, zip code 120024, Buzău, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: barcanuelena@yahoo.com

Abstract

The recent study was conducted at the Vegetable Research and Development Station Buzau, the unit has a number of 214 pepper genotypes in various stages of breeding. A number of 8 accession who demonstrated genetic stability and adequate for breeding were chosen for this study. The 8 accession whom are retained for evaluation are: A 24, A 25, A 26, A 54, A 55, A 56, A 57, A 58. These accession are characterized by a dwarf port and can be grown in pots as they are, in particular, appreciated like ornamental plants, having a long growing season and nice, decorative foliage and miniature fruits. Fruits can be eaten fresh throughout the whole year if pots are kept indoor or in a greenhouse during winter period. The lifetime of these accession is much longer than the varieties with large fruit, like bell pepper and long pepper. Kept in a pot, they can vegetate for nearly two years if pruning is made to stimulate new growth, knowing that pepper is a plant that blossoms and bears fruit continuously. New accession obtained have a distinct visible character like the shape and color of fruit:A 24, A 56 and A 58 presents red at fruit maturity stage, A 26 and A 54 orange, cultivars A 25 and A 55 have yellow fruits, and A 57 has fruit who passes through three stages color: purple, red and at physiological maturity becomes dark red. Researches will continue with patenting and extenteding in culture the new accession. The purpose of these research was to identify pepper genotypes, evaluating and entering them into germplasm of Vegetable Research and Development Station Buzau to be used for breeding.

***Key words**: Capsicum frutescens, germplasm, ornamental, chilli.*

INTRODUCTION

”Economicaly speaking, pepper crop represents one of the most important crop national and world wide due to high yields and best sales price” (Luchian, 2015). Despite that, chilli peppers, are not intensively cultivated in România and there are insufficient researches nationwide. Hot peppers are rather grown in small areas, predominantly in the farm system. In 1996, species has been taken in studies for intensive cultivation at Vegetable Research and Development Station Buzău. It has been taken a great care constructing a germplasm collection. "Considerable diversity exist in *Capsicum* L. germplasm for fruit and leaf shape and size, as well as plant habit. This morphological diversity, together with diverse ripe fruit color and varying hues of green to purple and variegated foliar pigmentation, affords a myriad of opportunities to develop unique cultivars for ornamental applications" (Stommel, 2004).

Depending on the directions of use, peppers are structured in: cultivars for fresh consumption, for production of paprika, preserved and for ornamental purposes. "A breeding program involves several activities such as germplasm bank maintenance, evaluation of genetic diversity, selection of superior genotypes, progenitor’s selection, hybridization, and evaluation of segregating populations. These activities are necessary, in general, to develop new cultivars."(Rêgo, 2016).

The aims of these paper is to select and obtain suitable cultivars for growing in pots as well as having ornamental purposes. "An ornamental pepper is a plant that is grown mainly for its aesthetic value. The architecture of ornamental pepper cultivars have to be compact and attractive, making them suitable for decorative purpose. These peppers vary widely in leaf and fruit color and growth habit."(Rêgo, 2012).

MATERIALS AND METHODS

Vegetable Research and Development Station Buzău possess a number of 214 pepper genotypes at different stages of breeding, of which: *Capsicum anuum* - 77 genotypes, *C. baccatum* - 8 genotypes, *C. chacoense* - 36 genotypes, *C. chinense* - 18 genotypes, *C. eximium* - 21 genotypes, *C. frutescens* - 48 genotypes, *C. galapagoense* - 6 genotypes.

Eight representative asscessions were studied chosen from 48 genotypes of germplasm collection of *Capsicum frutescens,* as follows: A 24, A 25, A 26, A 54, A 55, A 56, A 57 and A 58. These accessions are genetically stable and transmit unaltered in descending all characteristics.

The breeding methods used to obtain these accessions were the following: intraspecific hybridization between homozygous accessions, and the hybrid population resulted were individually selected for six generations followed by the annual mass selection.

Each line was cultivated in greenhouse, having in study 100 plants of each accession Seedling were made in a greenhouse multiplier, sow in alveolar pallets in the middle of march.

Planting was made at the begining of may, a special care should be considered as a species requires a light, well drain soil. Planting scheme used was 70 cm between rows and between plants in the row distance 25 cm, the lenght of vegetation period is 180-221 days. Each genotype was analyzed in accordance with UPOV examination of distinctness, uniformity and stability and also by characteristics such as: height, plant diameter, number of branches, plant height, leaf characteristics (length, width, lenght petiole).

RESULTS AND DISCUSSIONS

On the following study were analyzed plant characteristics and it can be noticed that plant height various between 9.4 cm to 22.3 cm from A 24 to A 58. Diameter of crown plant was different from genotype to genotype which is between 18.5 cm to 31.4 cm at A 54 to A 58. Another observation made was stalk height, A 24 had the shortest height and the tallest was 6.3 cm at A 55. It was noticed that cultivars have strong shoots from the base lines, registering a number of 4 branches at A54 and 10 branches at A 56. More details can be found in table 1.

Table 1. Main characteristic of chilli plant

Accession	Plant high (cm)	Diameter of crown plant (cm)	Stalk high (cm)	Number of shoots
A 24	9.4	22.3	2.2	6
A 25	16.5	28.4	5.4	9
A 26	14.2	24.2	4.3	7
A 54	12.6	18,5	4.6	4
A 55	19.3	23.7	6.3	8
A 56	13.2	25.4	2.4	10
A57	13.4	26.8	4.2	7
A 58	22.3	31.4	5.1	5

Analyzing the main characteristics of the leaves it can be noticed that A 57 has tiny leaves averaging only 4,7 cm lenght and a width of 1,7 cm of the leaf. Accession 58 showed the highest leaf with 11.0 cm long, 5.8 cm wide and 5.1 cm long petiole, as seen in table 2.

Table 2. Main characters of chilli peppers leaves

Accession	Leaf length (cm)	Leave with (cm)	Petiole length (cm)
A 24	5.4	2.0	3.4
A 25	6.1	2.7	4.2
A 26	5.8	2.4	3.5
A 54	5.7	2.2	3.2
A 55	4.9	2.3	3.2
A 56	6.1	2.3	4.2
A57	4.7	1.7	3.0
A 58	11	5.8	5.1

Regarding the number of fruit plant A 57 has recorded more than 100 fruits/plant and A 58 has recorded a total of 31 fruits / plant with a much greatest weight of 4.3 g/fruit and the smallest value in terms of weight was registered by A 24 with 1.4 g/fruit. The lowest value of lenght fruit was recorded by A 24 with a 2.8 cm and A 54 shows fruit over 3.3 cm, see table 3.

Table 3. Main character of chilli peppers fruits

Accession	Number fruits/ plant	Total mass of fruits/plant (g)	Mass fruit (g)	Fruit length (cm)
A 24	53	74.2	1.4	2.8
A 25	55	176	3.2	3.2
A 26	51	163.2	3.2	2.9
A 54	38	110,2	2.9	3.3
A 55	53	100.7	1.9	2.6
A 56	60	132	2.2	3.2
A57	100	230	2.3	3.1
A 58	31	133.3	4.3	2.9

Visible elements of distinction based on fruit characteristic of these 8 genotypes are shown in figure 1.

Figure 1. Fruit aspect in different stages of maturity

Thus, accession 24, 56 and 58 shows red at fruit maturity stage. Accession 25 and A 55 shows yellow at fruit maturity stage, A 26 and A 54 shows orange-yellow fruit at maturity stage. A distinguish fruit color had genotype A 57 which shows fruits whom pass through three stages. In the first phenophase, developing and fruit setting, shows purple fruit, in phenophase second turns red, and in the third phenophase, corresponding to physiological maturity, the fruits are dark red. Crop plant detail can be shown in figure 2.

Figure 2. Crop plant detail

A laboratory test was made to determine the content of capsaicin for each cultivar. In figure 3 it can be seen that cultivars A 26, A 54 and A 55 shows a poor pungency and A 57 and A 58 are having a higher content capsaicin, feature a sharp pungency.

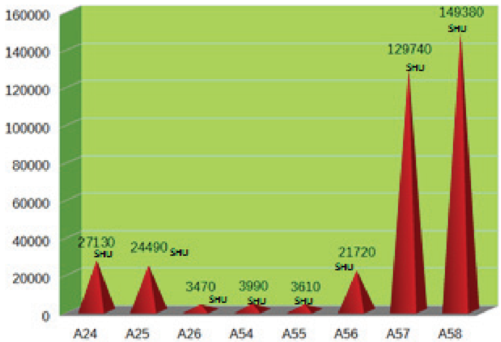


Figure 3. The capsaicin content (Scoville units-SHU)

CONCLUSIONS

Research were completed with the identification and recommendation of new eight distinct genotypes suitable for growing in pots, jardiniere, greenhouses and farms, especially in ornamental purposes.

Besides the interesting coloring fruit and plant port, an advantage is that fruits are edible. Therefore, A 57 will be patented and extended for cultivation with specific direction of use.

Also, the research ended with the establishment and evaluation of a valuable pepper germplasm collection which allows us obtaining new creations in the future.

REFERENCES

Luchian Viorica, Vînătoru C., 2016. Legumicultură. Editura „Alpha MDN”, Buzău.

Ramalho do Rêgo Elizanilda, Fortunato F.L.G., Nascimento Mayana, Finger F., 2012. Inheritance for earliness in ornamental peppers (*Capsicum annuum*), Acta horticulturae 961, 405-410.

Ramalho do Rêgo Elizanilda, Monteiro do Rêgo Mailson, 2016. Genetics and Breeding of Chili Pepper Capsicum spp. Production and Breeding of Chilli Peppers (*Capsicum* spp.). Springer International Publishing, 57-80.

Stommel J.R., Griesbach J.R., 2004. *Capsicum annuum* L.Tangerine Dream, HortScience 39(2), 448-449.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

DETERMINATION OF STRENGTH PROPERTIES FOR MECHANICAL HARVEST OF ROCKET (*ERUCA VESICARIA*)

Deniz YILMAZ, Mehmet Emin GOKDUMAN

Süleyman Demirel University, Agriculture Faculty,
Agricultural Machinery and Technologies Engineering Department,
tel. 02462118503, Doğu Campus, Isparta, Turkey

Corresponding author email: denizyilmaz@sdu.edu.tr

Abstract

Rocket (Eruca vesicaria) is a vegetable from the family Turpentaria (Brassicaceae) that eats leaves as a salad. Although rocket vegetable to produce small areas our country, it has started to make production in large and larger areas in recent years. This study aimed to determine the strength of Rocket (Eruca vesicaria) specifications for mechanical harvesting. For this purpose, properties as the maximum force, stress in the maximum force point, work at maximum force point, shearing force, deformation at maximum force, bioyield force, and shearing stress of rocket (Eruca vesicaria) stalk, leaf and root have determined. Average values for maximum force, stress, work to maximum force and deformation in maximum force were determined as 4.820 N, 0.474 MPa, 0.015 J and 22.149 mm, respectively. The shearing force and shearing stress were found to be as 2.150 N and 0.219 MPa, respectively. Average values for bioyield force were determined to be 3.856 N. These features can be used in determining the design and operating conditions for the mechanical harvester cutting blade.

Key words*: Rocket (Eruca vesicaria), strenght properties, mechanical harvesting.*

INTRODUCTION

'Rocket' is a common name used for some species in the family Brassicaceae that have a pungent aroma and a sharp taste (Figure 1). They are native to the Mediterranean and Near East, and they possibly acquired their original common name from the Lat-in-speaking Roman citizens who in-habited this area. The common name and many of its derivatives, including rughetta, rucola, roquette and others, most likely descended from the Latin word roc meaning harsh or rough (Pignone, 1997). Common names currently used to describe these species include roquette, rucola, arugula and rocket. As with all common names, the choice of common name varies with ethnicity, location and language group.
Rocket is traditionally grown in Italy, Portugal, Egypt, and Turkey (Bianco et al., 1997; Mohamedien, 1995; Pimpini et al., 1997; Silva Dias, 1997; Tuzel, 1995), it has also been successfully investigated as a new crop for Indiana and US Midwest (Morales et al., 2002), where it can be cultivated in open field and protected areas. In the past years, rocket has increasingly become popular also in the Central Europe.

Figure 1. Rocket (*Eruca vesicaria*) plant

It has 23,000 (1000 ha) of farmland in Turkey. 3.4 percent of this area (809,000 ha) used for vegetable production. Vegetable production has been increasing in recent years. According to 2015 data; the rocket production is 9110 tons, while tomato takes place on top with 12,615,000 tons in production volume of about 30 million tons.
The vegetable mechanization is conducted by hand in Turkey. Mechanization is needed due to the increase in production area.
The recent studies focused on chemical, herb and oil properties of rocket (*Eruca vesicaria*)

(Doležalová et al., 2013; Nurzyńska-Wierdak R., 2006). However, studies on strength properties of rocket are limited. This study covers determination of maximum force, stress in the maximum force point, work at maximum force point, shearing force, deformation at rupture force, bioyield force, shearing stress of rocket (*Eruca vesicaria*) stalk, leaf and root.

MATERIALS AND METHODS

For this study, rocket (*Eruca vesicaria*) plants were harvested by hand from the experimental field in Suleyman Demirel University, Isparta, Turkey.
Diameter and cross-sectional area of the experimental samples were measured before the shearing tests. Moisture content of the plants was determined at harvest time. Specimens were weighed and dried in an oven at 102°C for 24 h and then reweighed (ASABE, 2006). It was provided concise but complete information about the materials and the analytical and statistical procedures used.
A universal testing machine (LF Plus, UK) with a 500 N load cell and a computer-aided cutting and picking apparatus (Figures 2, 3) was used to measure the strength properties of the rocket plant. Knife material was hardened iron. All the tests were carried out at a speed 0.8 mm s^{-1}, and data were recorded at 10 Hz. All data were analysed by nexygen software program.

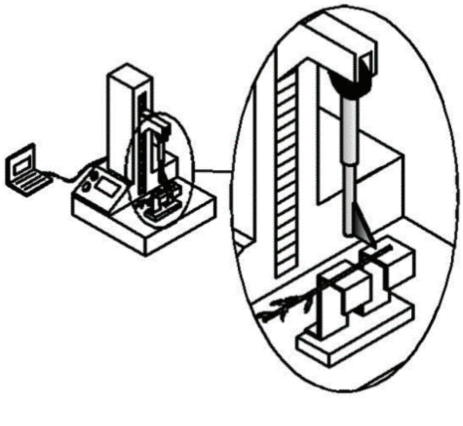


Figure 2. Cutting system

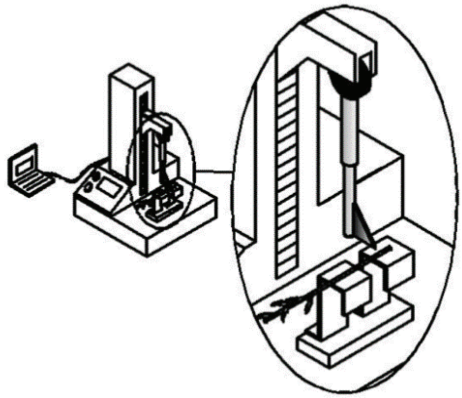


Figure 3. Picking system

The shearing forces on the load cell with respect to knife penetration were recorded by computer (Ozbek et al., 2009).
The shearing stress in N/mm^2 was calculated using the equation of Shahbazi et al., 2012:

$$\tau = \frac{F_{s\max}}{A}$$

Where F_{smax} is the maximum shearing force of the curve in N, and A is the area of the stalk at the deformation cross-section in mm^2.

The rocket plants were attached to the apparatus from its stalks (Figure 4). The shearing tests were conducted with 0.8 $mm.s^{-1}$ knife speed progress (Simonton, 1992).

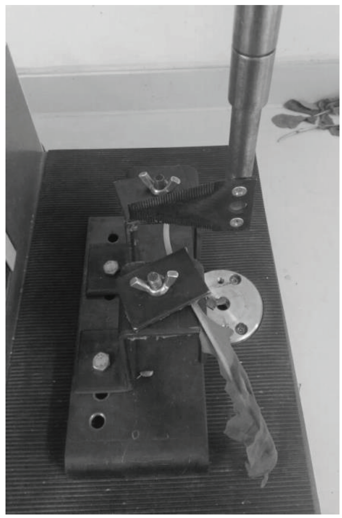
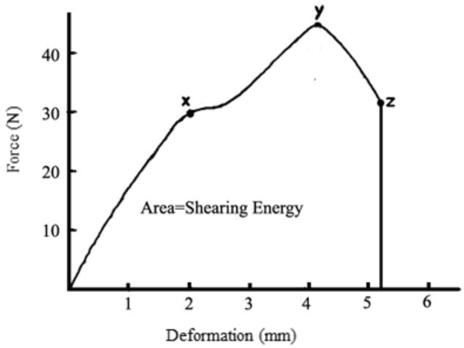


Figure 4. Measuring the cutting of rocket (*Eruca vesicaria*) plant

Picking force can be defined as the force required to separate leaf from ovary point (picking force of leafs). The load cell of the machine was then pulled upward to determine the picking force of the rocket leafs (Figure 5).

Figure 5. Measuring the picking force of rocket leaf

Bioyield force, shearing force, bending stress, shearing stress, and shearing deformation were calculated from the force-deformation curves at the inflection point as defined by ASAE Standard (1985). S368.1 (ASAE Standards, 1985) was obtained from all curves (Figure 6). The energy of shearing was determined as the area under these curves (Chen et al., 2004; Srivastava, 2006).



Note. Labels on the graph indicate the following points:

x – bioyield force, y – maximum force, z – shearing force (Liu, 2012).

Figure 6. Typical force-deformation curve of rocket (*Eruca vesicaria*) stalk during shearing loading

RESULTS AND DISCUSSIONS

Moisture content of the plants was determined as 89% at harvest time and all tests were conducted at harvest moisture. The strength measurements of rocket stalks are given in Table 1.

Table 1. Average strength properties of rocket stalk

	Maximum force (N)	Bioyield force (N)	Shearing force (N)	Stress in maximum force (MPa)	Energy in maximum force (J)	Shearing stress (MPa)	Shearing deformation (mm)	Area (mm^2)
Stalk	4.820	3.856	2.510	0.474	0.015	0.219	22.149	10.534
Standard deviation	2.940	1.256	2.544	0.154	0.012	0.133	2.509	6.124

The maximum force was observed as 4.820 N at rocket stalk. The bioyield force of 3.856 N was observed at Stalk. Shearing force is one of the most important plant characteristics affecting plant harvesting. If the weight of the plant is known, the shearing force and the shearing height can be used to determine the speed of the blade to be used in harvesting (Igathinathane et al., 2010; Taghijarah et al., 2011). The maximum shearing force was observed as 2.510 N at Stalk. The maximum stress value (0.474 MPa) was observed at Stalk. The energy at maximum force was found to be as 0.015 J. Deformation has an important place among the strength characteristics of the plant. The maximum shearing deformation (22.149 mm) was observed at Stalk. The average cross-sectional area of rocket was determined as 10.534 mm^2 at harvest moisture (89.9 %). The strength measurements of rocket leaf are given in Table 2.

Table 2. Average strength properties of rocket leaf

	Maximum force (N)	Bioyield force (N)	Shearing force (N)	Stress in maximum force (MPa)	Energy in maximum force (J)	Shearing stress (MPa)	Shearing deformation (mm)
Leaf	6.233	4.986	3.659	0.235	0.024	0.114	7.477
Standard deviation	3.044	1.563	2.088	0.102	0.017	0.085	2.623

The maximum force required to separate leafs from stalk was determined as 6.233 N. As a function of the maximum force the bioyield force was found to be 4.986 N. Lower shearing forces required for mechanical harvesting leads to savings in power and energy usage. Leaf shearing force of rocket observed 3.659 N is higher than stalk shearing force. The maximum stress in maximum force value (0.235 MPa) was observed at leaf. The energy at maximum force was found to be as 0.024 J.

Average shearing deformation value for rocket leaf was determined as 7.447 mm. The strength measurements of rocket root are given in Table 3.

Table 3. Average strength properties of rocket root

	Maximum force (N)	Bioyield force (N)	Shearing force (N)	Stress in maximum force (MPa)	Energy in maximum force (J)	Shearing stress (MPa)	Shearing deformation (mm)	Diameter (mm)
Root	21.798	17.439	18.912	1.097	0.084	1.017	19.785	3.924
Standard deviation	8.207	6.566	11.642	0.652	0.047	0.713	3.708	0.845

The maximum force and shearing force are important design parameters for harvesters and they should be known for power requirement. Therefore, the maximum force and shearing force were determined as 21.798 and 18.912 N, respectively. The bioyield force of (17.438 N) was observed at root. The stress value in maximum force was found to be as 1.097 MPa. The energy at maximum force (0.084 MPa) was observed at root. Average shearing deformation value of rocket root was observed as 19.785 N. The average root diameter of rocket *Eruca vesicaria* was determined as 3.924 mm at harvest moisture (89.9 %)

CONCLUSIONS

This study was carried out to determine the strength properties of rocket plant (*Eruca vesicaria*) at leaf, stalk and root sections in the harvest moisture. Properties as the maximum force, bioyield force, shearing force, stress in maximum force, energy in maximum force, shearing stress, shearing deformation of rocket leaf, stalk and root have determined at moisture content of 89.9%.

The strength parameters measured at root section higher than that of the stalk and leaf sections.

The lowest values were found at rocket stalk. The strength parameters of stalk section should be considered for mechanical harvesting of rocket plant to provide data for the design machines for mechanized applications.

REFERENCES

ASABE Standards., 2006. Moisture measurement e Forages. St. Joseph, MI: American Society of Agricultural and Biological Engineers (ASABE). S358.2

Asae Standards, 1985. Compression test of food materials of convex shape. St. Joseph, Mich.: American Society of Agriculture Engineering, S368.1

Bianco V.V., Boari F., 1997. Up-to-date developments on wild rocket cultivation, In: Padulosi S, Pignone D (Eds.). Rocket: A Mediterranean crop for the world. Report of a workshop 13-14 December 1996, Legnaro (Padova), Italy, 41-49.

Chen Y., Gratton J. L., Liu J., 2004. Power requirements of hemp cutting and conditioning. Biosystems Engineering, 87(4), 417-424.

Dolezalova I., Duchoslav M., Dusek, K., 2013. Biology and Yield of Rocket. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 41(2), 530.

Igathinathane C., Womac A. R., Sokhansanj S., 2010. Corn stalk orientation effect on mechanical cutting. Biosystems engineering, 107(2), 97-106.

Mohamedien S., 1995. Conservation and utilization of rocket in Mediterranean countries. Rocket cultivation in Egypt, p. 61-62. In: Padulosi S (Ed.). Rocket Genetic Resources Network. Report of the First Meeting, 13-15 November 1994, Lisbon, Portugal.

Morales M., Janick J., 2002. Arugula: A promising specialty leaf vegetable, In: Janick J, Whipkey A (Eds.). Trends in new crops and new uses. ASHS Press, Alexandria, VA, p. 418-423.

Nurzyńska-Wierdak R., 2006. The effect of nitrogen fertilization on yield and chemical composition of garden rocket (*Eruca sativa* Mill.) in autumn cultivation. Acta Sci PolHortoru 5:53-63.

Ozbek O., Seflek A.Y., Carman K., 2009. Some Mechanical Properties of Safflower Stalk. Appl. Eng. Agric., 25: 619–625.

Pignone D. 1997. Present status of rocket genetic resources and conservation activities, In: Padulosi S. & Pignone D. (ed.). Rocket: a Mediterranean crop for the world. Report of a workshop, 13-14 December 1996, Legnaro (Padova), Italy. International Plant Genetic Resource Instute, Rome, Italy, 2-12.

Pimpini, F., Enzo M., 1997. La coltura della rucola negli ambienti veneti. Colture protette 4:21-32.

Shahbazi F., Nazari Galedar M., 2012. Bending and shearing properties of safflower stalk. Journal of Agricultural Science and Technology, 14(4), 743-754.

Silva Dias J.C., 1997. Rocket in Portugal: botany, cultivation, uses and potential, In: Padulosi S, Pignone D (Eds.). Rocket: A Mediterranean crop for the world. Report of a workshop 13–14 December 1996, Legnaro (Padova), Italy, 81-85.

Simonton W., 1992. Physical properties of zonal geranium cuttings. Trans. ASAE, 35(6): 1899-1904.

Srivastava A.K., Goering C.E., Rohrbach R.P., Buckmaster D.R., 2006. Engineering principles of agricultural machines (2nd ed.). American Society of Agricultural and Biological Engineers, St. Joseph, USA, 185.

Taghijarah H., Ahmadi H., Ghahderijani M., Tavakoli M., 2011. Shearing characteristics of sugar cane (*Saccharum officinarum* L.) stalks as a function of the rate of the applied force. Australian Journal of Crop Science, 5(6), 630.

Tuzel Y., 1995. Conservation and utilization of rocket in Mediterranean countries. Rocket growing in Turkey, In: Padulosi S (Ed.). Rocket Genetic Resources Network. Report of the First Meeting, 13-15 November 1994, Lisbon, Portugal, 58-60.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF GRAFTING ON PRODUCTION AT SOME GRAFTED EGGPLANTS

Mădălina DOLTU[1], Marian BOGOESCU[1], Dorin SORA[1], Vlad BUNEA[2]

[1]Research and Development Institute for Processing and Marketing of Horticultural Products – Horting, 1A Intrarea Binelui Street, District 4, 042159, Bucharest, Romania
[2]Central School, Bucharest, Romania, 3-5 Icoanei Street, District 2, 20451, Bucharest, Romania

Corresponding author email: doltu_mada@yahoo.com

Abstract

The scientific research is concerned continuously of achieving some high value biological creations, resistant or tolerant to diseases and pests, high productivity, high quality fruits; these goals are obtained by grafting. The eggplants are plants that can be grafted onto different rootstocks. The grafted seedlings transmit to crops quality, productivity, resistance to pests and diseases from soil, tolerance to abiotic stress factors, optimal absorption of water and nutrients, vigour. The study was conducted in a greenhouse of the Horting Institute Bucharest and it has followed the rootstock influence on the production quality at some grafted eggplants. The biological material has consisted of import F1 hybrids, eggplant scions (''Black Pearl'' and ''Classic'') and rootstocks (''Emperador'' and ''Torpedo'') commonly used in Romania for grafting of eggplants. It were determined form index (FI), form (F), weight/fruit (W), marketable production (MP), humidity (H) – gravimetric method, soluble dry matter (SDM) by refractometry method using ABBE refractometer, total sugar (TS) by Bertrand method. The results show that the grafting had influenced some aspects of the eggplant production. The conclusions of the researchers from the Horting Institute are in respect with some conclusions of the foreign researchers, but more researches are required in this domain to highlight the grafting effect on some aspects concerning production of grafted vegetables.

***Key words**: grafting, yield, Solanum melongena L., quality.*

INTRODUCTION

The scientific research is concerned continuously of achieving some high value biological creations, resistant or tolerant to diseases and pests, high productivity, high quality fruits; these goals are obtained by grafting (Doltu, 2007).

The vegetable grafting has been used since the early decades of the XIX century in the countries from the Far East and it is considered to be an ecological way to reduce the attack of pathogens and pests of soil (fungi, bacteria and nematodes) which, particularly in intensive culture conditions, produce considerable production loss and abandonment of some cultures.

This planting material is a biological alternative for replacing polluting chemicals used to disinfection of the soil (Echevarria et al., 2004). The grafted seedlings print quality, productivity, resistance to diseases (*Fusarium* spp., *Verticillum* spp.) and pests (nematodes) of soil (Bogoescu et al., 2008).

The grafting print resistance to pathogens and pests of soil, tolerance to abiotic stress factors, improves absorption about water and nutrients, increase vigour to scion (King et al., 2010 and Lee, 1994).

Some researchers belive that the results concerning the fruit quality obtained from grafted plants are contradictory (Davis et al., 2008).

Çürük et al., 2009 had investigated the grafting influence on eggplants, noting that the fruit average weight is significantly influenced by grafting.

The results concerning the grafting influence on vegetable crops require more researches in this domain for to highlight the grafting effect on some aspects concerning production of grafted vegetables.

MATERIALS AND METHODS

The biological material used in research has consisted from eggplant scions and rootstocks commonly used in Romania to grafting of eggplants.

The scions were F1 hybrids of eggplants, 'Black Pearl' (Enza Zaden, Netherlands), 'Classic' (Clause Vegetale Seeds, France) and F1 hybrids of rootstocks 'Emperador' (Rijk Zwaan, US) and 'Torpedo' (Ramiro Arnedo Semillas, Spain).

The ungrafted and grafted eggplant seedlings had been obtained into specialized greenhouse for production of seedlings from Laboratory of Protected Cultures, Institute Horting Bucharest.

The grafting technique have consisted in more stages: sowing (scion and rootstock), preparing for grafting, grafting itself, introduction of grafted plants in polyethene tunnel for callus forming, transferring of seedlings in greenhouse for grower and maintenance according with the 'Classic' technology (Bogoescu M. et al., 2008).

The experimental variants were made up from lots of ungrafted (control) and grafted plants:

- ungrafted plants (control):
  - 'Black Pearl' (V1)
  - 'Classic' (V2)
- grafted plants (scion x rootstock):
  - 'Black Pearl' x 'Emperador' (V3)
  - 'Classic' x 'Emperador' (V4)
  - 'Black Pearl' x 'Torpedo' (V5)
  - 'Classic' x 'Torpedo' (V6)

The experimental lots with ungrafted and grafted eggplants were set up at 27/06/2015 at greenhouse of glass and maintained according with the specific technologies (Figure 1, a and b).

The experience was made up of 96 plants/V1 or V2 (24,000 plants ungrafted/ha) and 72 plants/V3 or V4, V5 and V6 (18,000 grafted plants/ha). It was placed by the randomized block method in 4 repetitions (24 plants/ repetition V1, V2 and 18 plants/repetition V3, V4, V5 and V6).

The observations, the biometric determinations and the biochemical analyzes on eggplant fruits were performed in the laboratories of the Horting Institute on biological samples harvested to consumer maturity.

a) After planting

b) During the vegetative period

Figure 1. Experimental lots with grafted eggplants cultivated into greenhouse

It were determined form index (FI), form (F), weight/fruit (W), marketable production (MP), humidity (H) – gravimetric method, soluble dry matter (SDM) by refractometry method using ABBE refractometer, total sugar (TS) by Bertrand method.

The experience was carried out under conditions of extreme temperatures, very high in the summer of 2015; these temperatures were a calamity for vegetable cultures.

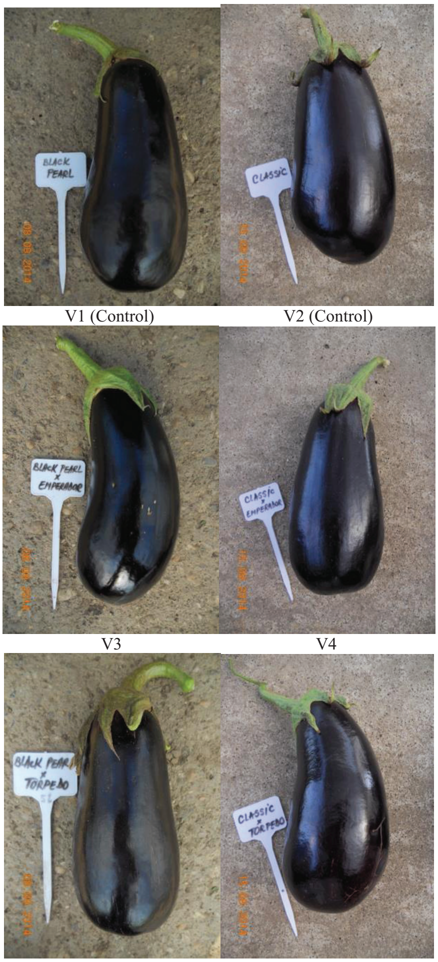
RESULTS AND DISCUSSIONS

The results concerning some observations, biometric determinations and biochemical analyzes performed on eggplant fruits are shown in Table 1. The grafting did not affect the fruit form.

The grafted and ungrafted plants have had the fruits with same form, elongated eggplants (Figure 2).

Table 1. Data concerning some fruit characteristics from eggplant variants

Variants	Biometric determinations			Biochemical analyzes		
	FI / F	W (g/fruit)	MP (kg/ha)	SDM (ºR)	TS (%)	H (%)
V1 (Control)	2.25 / elongate	353	50,230	4.60	1.50	92.10
V2 (Control)	2.30 / elongate	305	50,200	4.50	1.30	92.10
V3	2.25 / elongate	273	61,400	5.00	1.27	94.17
V4	2.28 / elongate	255	58,120	4.60	1.25	93.90
V5	2.27 / elongate	281	58,390	4.75	1.39	94.05
V6	2.28 / elongate	283	56,980	4.63	1.24	93.98



V1 (Control) V2 (Control)
V3 V4
V5 V6

Figure 2. Eggplant form, elongate, V1,V2, V3, V4, V5, V6

The fruit weight was not significantly influenced by the grafting. At some grafted variants (V3 and V6), 'Emperador' and 'Torpedo'
rootstocks had determined obtaining of fruits with weighing less than the ungrafted variants, control (V1 and V2) and another grafted variants (V5 and V4) had determined obtaining of fruits with weighing more bigger than the ungrafted variants, control (V1 and V2).

There is a direct linear correlation between variants and the average marketable production. The determination coefficient shows that for production (kg/ha), the correlation is very significant, $r^2 = 1$ (Figure 3).

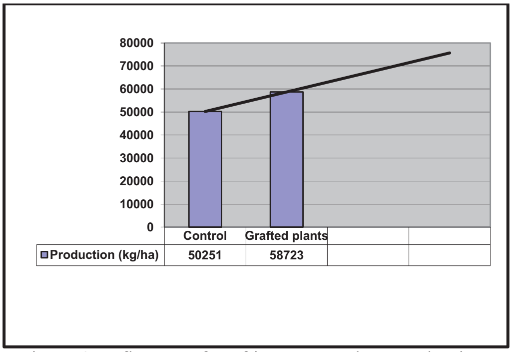


Figure 3. Influence of grafting on eggplant production

The fruit color harvested at consumer maturity was not influenced by the grafting; such as the fruits from grafted plants had same color as the fruits from ungrafted plants, control.

The differences were insignificant about the content some biochemical components.

About SDM, the grafted variants (V2, V3, V4, V5 and V6) have had slightly higher values compared with the ungrafted variants (V1 and V2). The S content was higher in ungrafted variants (V1 and V2) compared with the grafted variants (V2, V3, V4, V5 and V6), but the differences were not significant.

Bogoescu and Doltu, 2015, show in a specialty paper that ungrafted eggplants have a higher carbohydrate content (2.54–2.97%) compared with the grafted eggplants (1.92–2.00%) cultivated under same biotope and technological conditions.

About H, the ungrafted variants (V1 and V2) has had same value and slightly lower than the grafted variants (V2, V3, V4, V5 and V6).

The results obtained by some experts (Moncada et al., 2013) about the yield and the quality of some grafted eggplants are contradictory.

CONCLUSIONS

The extreme temperatures, very high from the summer of 2015, considered a calamity, led to a strong abiotic stress on plants during the vegetable period; thus, the results about eggplant production were inconclusive.

The grafting effect on eggplants it was insignificant in the climatic conditions of 2015.

The fruits of the grafted plants have form, color and weight as the fruits than ungrafted plants.

At the grafted variants, the soluble dry matter content was higher, the sugar content was lower and the humidity was higher than in the ungrafted variants, but the differences were not significant.

The rootstock and scion plants are compatible regarding the fruit quality.

The results obtained by the research in 2015 concerning the grafting effect on the eggplant production are not relevant; thus it requires further researches on these issues.

The conclusions of the researchers from the Horting Institute are in keeping with some conclusions of the foreign researchers, but are required more researches in this domain for to highlight the grafting effect on some aspects concerning production of grafted vegetables.

ACKNOWLEDGEMENTS

This research was conducted within the internal thematic plan of the Horting Institute Bucharest from 2015, project supported from the funds of the institute.

REFERENCES

Bogoescu M., Doltu M., Sora D., Iordache B., 2008. Results on establishing the technology for obtaining the tomatoes grafted seedlings designed for greenhouses. Bulletin UASVM Horticulture Cluj-Napoca, vol. 65(1), 147-152.

Bogoescu M., Doltu Mădălina, 2015. Effect of Grafting Eggplant (*Solanum melongena* L.) on its Selected Useful Characters. Bulletin UASVM Horticulture Cluj-Napoca, vol. 72(2), 313-317.

Çürük S., Dasgan H. S., Mansuroglu S., Kurt S., Mazmanoglu M., Antaklı O., Tarla G., 2009. Grafted eggplant yield, quality and growth in infested soil with *Verticillium dahliae* and *Meloidogyne incognita*. Pesq. agropec. bras., Brasília, vol. 44(12), 1673-1681.

Davis A.R., Perkins-Veazie P., Hassell R., King S.R., Zhang X., 2008. Grafting effects on vegetable quality. HortScience vol. 43, 1670-1672.

Doltu M., 2007. Stabilirea tehnologiei pentru producerea de rasadurilor altoite de tomate. Editura Agris Bucuresti, Revista Horticultura nr. 9, 4.

Echevarria P.H., Rodriguez A., Vivaracho S.M., Vallejo A.D., 2004. Influence of rootstocks and soil treatment on the yield and qulity of greenhouse-grown cucumbers in Spain – Acta Horticulture vol. 663, 403-408.

King S.R., Davis A.R., Zhang X., Crosby K., 2010. Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae. Sci. Hort. vol.127, 106-111.

Lee J.M., 1994. Cultivation of grafted vegetables I: current status, grafting methods and benefits. Hortscience vol. 29, 235-239.

Moncada A., Miceli A., Vetrano F., Mineo V., Planeta D., D'Anna F., 2013. Effect of grafting on yield and quality of eggplant (*Solanum melongena* L.). Scientia Horticulturae, Advances in Vegetable Grafting Research vol. 149, 108-114.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE EFFECTS OF DIFFERENT FERTIGATION TREATMENTS ON YIELD AND NUTRIENT UPTAKE OF WATERMELON PLANTS GROWN AS SECOND CROP IN CUKUROVA REGION

Ahmet DEMIRBAS

Cumhuriyet University, Vocational School of Sivas,
Department of Crop and Animal Production, Sivas, Turkey

Corresponding author email: ademirbas@cumhuriyet.edu.tr

Abstract

The present study was conducted to investigate the effects of different fertigation treatments (25, 50, 75 and 100 %) on yield and nutrient uptake of watermelon as the second crop and to compare with conventional practices. Experiments were conducted over the experimental fields of Çukurova University, Agricultural Faculty Soil Science and Plant Nutrition Department under field conditions. Experiment was conducted in randomized blocks split plots design with three replications. A total of 160 kg ha^{-1} nitrogen (N) as ammonium sulphate, 100 kg ha^{-1} phosphorus (P) as MKP and 200 kg ha^{-1} potassium (K) as KNO_3 were applied. Seedlings were planted in peat/perlite mixture (1:1 V/V) and transplanted to experimental plots following with wheat harvest in June. The watermelon plants were irrigated 9 times during the growing season in one week intervals. Current findings revealed that 75% fertigation treatment (120 kg ha^{-1} N, 75 kg ha^{-1} P, 150 kg ha^{-1} K) had the greatest yield (48.38 t ha^{-1}). Also, it increased N (%3.78), P (%0.31), Zn (45.7 mg kg^{-1}), Mn (43.1 mg kg^{-1}) and Cu (17.6 mg kg^{-1}) contents of plants.

Key words: *Fertigation, watermelon, yield, nutrient uptake.*

INTRODUCTION

Watermelon production had a significant place in world agriculture. Worldwide, watermelon is produced over 1.8 million hectares and annual production is around 29.7 million tons. Turkey has the second place worldwide after China in melon and watermelon production. Despite decreasing production lands in Turkey, annual production is increasing with incresing yields through proper cultural practices (Taşkaya and Keskin, 2004). The watermelon (*Citrullus lunatus* (Thunb.) is commonly grown in Turkey and fruits are consumed. Annual watermelon production of Turkey in 2008 from 123.000 ha was 3.5 million tons. In annual watermelon production Turkey, Çukurova with 678.73 thousand tons had the first place (Anonymous, 2008).
Just because of availability for production over large areas, easy marketing and high return rates per unit area; watermelon is also commonly produced in high or low tunnels (Yetişir and Sarı, 2004).
Watermelon has quite fast growing rate, short vegetation period and contains about 90-92% water. Therefore, irrigation is a must for high yield levels (Miller, 2002). Watermelon requires more frequent irrigation intervals because of excessive evaporations and low precipitation rates throughout the growing season (Doorenbos and Kassam, 1979).
Therefore, drip irrigation recommended for watermelon irrigation (Srinivas et al., 1989). Fertilizers are usually applied through drip lines together with irrigation water. This practice is called fertigation (Bar-Yosef, 1991). It is a new agricultural technique that provides fertilizer and water concurrently (Majahan and Singh, 2006; Castellanos et al., 2012). In this way, it is possible to control timing, amount and concentration of fertilizers (Hagin et al., 2002). It can provide water and fertilizer in a timely and correctly and increases nutrient uptake. Through fertigation, fertilizer contact with soil is minimized, fertilizers are directly applied to plant root zones and significant savings are achieved in water and fertilizers (Mohammad and Zuraiqi, 2003; Beyaert et al., 2007).
Fertigation is able to meet nutrient requirements of almost all plants, thus almost entire plant water requirement of watermelon is met over the low yield fields poor in nutrients

and in places with irregular precipitations (Fernandes and Prado, 2004).
In this study, effects of different fertigation treatments on plant yield and nutrient uptake of watermelon grown as a second crop in Çukurova region with a semi-arid climate were investigated.

MATERIALS AND METHODS

The present research was conducted in summer season of 2012 over the experimental fields of Çukurova University Agricultural Faculty Soil Science and Plant Nutrition Department under field conditions. Soil characteristics are provided in Table 1.

Table 1. Physical and chemical characteristics of experimental soils.

Soil Property	Depth (0-30 cm)
pH (H_2O)	7.59
Lime (%)	24.1
Salt (%)	0.039
Organic matter (%)	1.30
Texture	CL
Total N (%)	0.10
Available P (kg ha^{-1})	24.2
Available K (kg ha^{-1})	1010.5
Available Fe (mg kg^{-1})	4.01
Available Mn (mg kg^{-1})	1.12
Available Zn (mg kg^{-1})	0.51
Available Cu (mg kg^{-1})	0.31

Experiments were conducted in randomized blocks split plots experimental design with 3 replications. Together with conventional method (0% fertigation), different fertigation doses as of 25% (40 kg N ha^{-1}, 25 kg P ha^{-1}, 50 kg K ha^{-1}), 50% (80 kg N ha^{-1}, 50 kg P ha^{-1}, 100 kg K ha^{-1}), 75% (120 kg N ha^{-1}, 75 kg P ha^{-1}, 150 kg K ha^{-1}) and 100% (160 kg N ha^{-1}, 100 kg P ha^{-1}, 200 kg K ha^{-1}) were experimented.
Just based on fertigation doses, remaining fertilizers were applied to soil with conventional method as follows:

- 25% fertigation - 75% conventional,
- 50% fertigation - 50% conventional,
- 75% fertigation - 25% conventional and
- 100% fertigation - 0% conventional.

In conventional method (0% fertigation) 160 kg N ha^{-1}, 100 kg P ha^{-1}, 200 kg K ha^{-1} were also applied. P and K were applied at planting and N was applied in 3 portions. Irrigations were applied in the same fashion and same number of irrigations was performed. N was applied in ammonium sulphate form, P in MKP and K in KNO_3 form.
Crisby watermelon cultivar was used as the plant material. Seedlings were planted in peat:perlite (1/1 V/V) medium under greenhouse conditions. Then the seedlings were transplanted to field conditions as the second crop after wheat harvest on 15th of June. Each plot had 4 rows and each row had 4 plants. Therefore, each treatment had 16 plants. Row spacing was 1.80 m, on-row plant spacing was 1 m and total plot size was 16.2 m^2. There was 2 m spacing between the adjacent plots to prevent interactions among treatments. A total 9 irrigations were performed throughout the experimental period in 1 week intervals. Water was not supplied for 15 days during shoot elongation period. Leaf samples were taken from watermelon plants at the beginning of flowering. Samples were ground in a plant grinder and N content was determined with modified Kjeldahl method (Bremner, 1965). For P, K, Fe, Mn, Zn and Cu contents, 0.200 g plant samples were ashed at 550 ºC for 5.5 hours in an ash oven. Then the samples were supplemented with 1/3 HCl and distilled water. Readings were performed in resultant extract at P 882 nm in a UV-spectrophotometer (Murphy and Riley, 1962). K, Fe, Mn, Zn and Cu contents were determined with an Atomic Absorption Spectrophotometer (AAS) (Güzel et al., 1992).
Watermelon plants were harvested 77 days after transplantation into field. Following the harvest, yields were determined.
Experimental results were subjected to analysis of variance (ANOVA) by using SPSS 22.0 Windows software. The difference between treatments means were tested with Tukey's test.

RESULTS AND DISCUSSIONS

The effects of different fertigation doses on yields of watermelon plants are presented in Figure 1. Current findings revealed that 75% fertigation treatment had the greatest effect on yield of watermelon plants (48.38 t ha^{-1}). It was followed by 100% fertigation treatment with a yield of 48.14 t ha^{-1}. Bhat et al (2007) carried out a study with data palm plants between the years 1996-2006 and applied 4 different fertigation doses (25, 50, 75 and 100 of recommended fertilizer dose). Researchers reported the greatest yield for 75% fertigation treatment (75:13.5:87.7 g N, P, K year^{-1}) with a yield of 37.21 t ha^{-1}. The 0% fertigation dose (conventional fertilizer application) with a yield of 44.29 t ha^{-1} had higher yield than 25 and 50% fertigation treatments and the differences from 75% fertigation treatment were not significant. The yield of watermelon plants grown as second crop varied between 27.46–48.38 t ha^{-1}. In previous studies, based on climate conditions and water management systems, watermelon yields were reported as between 50 t ha^{-1} and 80 t ha^{-1} (Srinivas et al., 1989; Gündüz et al., 1996; Simsek et al., 2004).

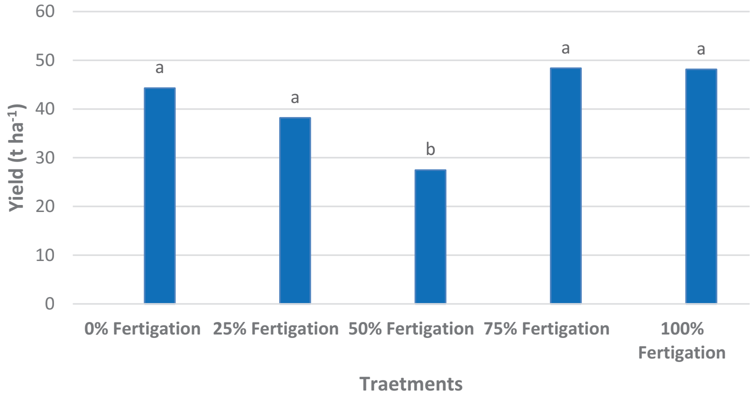


Figure 1. Effects of different fertigation doses on yields of watermelon plants (t ha^{-1})

Considering the effects of treatments on N contents of watermelon plants (Table 2), it was observed that 75% fertigation doses had the greatest N content with 3.78%. The 0% fertigation doses (conventional method) had the lowest N content with 3.43%. As compared to 0% fertigation dose, other treatments increased N contents of plants more. Similar to N contents, 75% fertigation treatments had the greatest P content with 0.31% and 0% fertigation treatment had the least P content with 0.25%. Such a case may be resulted from direct application of plant nutrients, especially hard-to-transport phosphorus to soil through fertigation. Again as it was in N and P contents, all fertigation treatments, except for 0%, increased plant K contents. Among the fertigation treatments, 50% treatment had the greatest K content (3.62%), because N promoted only the vegetative growth and eased the uptake of other nutrients like P and K (Riley and Barber, 1971; Soon and Miller, 1977). Such a synergic impact of nitrogen on other plant nutrients also supports the P uptake within the root zone (Drew, 1975; Anghinoni and Barber, 1988). Potassium (K) and nitrogen (N) are the mostly used nutrients in watermelon (Grangeiro and Cecílio Filho, 2004; Vidigal et al., 2009; Silva et al., 2012). When these nutrients were taken through fertigation, they are distributed uniformly and thus improve fruit quality and yield levels (Hochmuth, 1992), and reduce production costs and prevent various environmental problems.

Table 2. Effect of different fertigation treatments on N, P and K contents of watermelon plants (%)

Treatments	**N**		**P**		**K**	
0% Fertigation	3.43	±0.02 c	0.25	±0.02 c	3.13	±0.02 d
25% Fertigation	3.75	±0.18 a	0.27	±0.00 bc	3.21	±0.04 cd
50% Fertigation	3.53	±0.06 bc	0.29	±0.01 ab	3.62	±0.15 a
75% Fertigation	3.78	±0.00 a	0.31	±0.00 a	3.52	±0.15 ab
100% Fertigation	3.70	±0.13 ab	0.29	±0.01 ab	3.38	±0.13 bc

$P<0.05$

Considering the effects of different fertigation treatments on microelement content of watermelon plants, it was observed that the greatest Fe content was obtained from 50% fertigation treatment with 135.5 mg kg^{-1} and it was followed by 0% fertigation treatment (conventional method) with 129.1 mg kg^{-1} (Table 3).

Table 3. Effect of different fertigation treatments on Fe, Zn, Mn and Cu contents (mg kg^{-1})

Treatments	Fe		Zn		Mn		Cu	
0% Fertigation	129.1	±1.62 ab	36.5	±0.72 c	36.4	±0.40 b	14.5	±1.71 c
25% Fertigation	118.7	±3.74 c	41.3	±0.19 b	36.2	±0.14 b	15.7	±1.06 a-c
50% Fertigation	135.5	±3.60 a	41.1	±0.36 b	37.4	±3.31 b	15.6	±0.82 bc
75% Fertigation	125.1	±2.12 bc	45.7	±2.35 a	43.1	±3.83 a	17.6	±0.34 a
100% Fertigation	108.2	±5.23 d	41.4	±1.28 b	35.9	±1.06 b	17.3	±0.33 ab

P<0.05

The lowest Fe content was obtained from 100% fertigation treatment (108.2 mg kg^{-1}). Considering the N contents, similar to N, P and K contents, the greatest value was observed in 75% fertigation treatment (45.7 mg kg^{-1}) and 0% fertigation treatment (36.5 mg kg^{-1}) did not have significant effects on Zn contents as compared to other treatments. Again 75% fertigation treatment had the greatest Mn content (43.1 mg kg^{-1}) and Cu content (17.6 mg kg^{-1}). The relations between the parameters are presented in Table 4.

Table 4. Correlation among variables tested in the experiment

	Yield	N	P	K	Fe	Zn	Mn
Yield							
N	0.185						
P	0.097	-0.392					
K	-0.360	-0.444	0.754**				
Fe	-0.584*	0.361	-0.090	0.322			
Zn	0.153	-0.181	0.869**	0.629*	-0.155		
Mn	0.231	0.150	0.609*	0.397	0.220	0.723**	
Cu	0.311	-0.460	0.800**	0.456	-0.467	0.759**	0.464

*Significant at P<0.05
**Significant at P<0.01

Positive correlation was determined between P and K, Zn, Cu concentrations (p<0.01) and Mn concentrations (p<0.05). Also between Zn and Mn, Cu concentrations (p<0.01) positive correlation was determined. Generally, the other relations were found to be insignificant (p>0.05).

CONCLUSIONS

Considering the entire results of the present study, it was concluded that nutrient supply through fertigation significantly improved yields and N, P, K, Zn, Mn and Cu contents of watermelon plants as compared to conventional method of nutrient supply (0% fertigation).

Especially 75% fertigation treatment (25% from the soil) had greater impacts on watermelon plants than the other fertigation treatments. In general, irrigation improves yields and yield components of watermelon plants grown in semi-arid climate conditions.

REFERENCES

Anghinoni I., ve Barber S.A., 1988. Corn root growth and nitrogen uptake as affected by ammonium placement. Agron. J. 80:799-802.

Anonymous, Bitkisel Üretim İstatistikleri, T.C. Başbakanlık Türkiye İstatistik Kurumu (TÜİK), Ankara, www.tuik.gov.tr (*Erişim Tarihi: 30/11/2008*).

Bar-Yosef B., 1991. Fertilization Under drip Irrigtion . In: Fluid Fertilizer Science and Technology (Eds. Palgrave, Derek A., Marcel Dekker) Inc. New, 285-329.

Beyaert R.R., Roy R.C., ve Coelho B.K.B., 2007. Irrigation and fertilizer management effects on processing cucumber productivity and water use efficiency. Can. J. Plant Sci. 87, 355–363.

Bhat R., Sujatha S., ve Balasimha D., 2007. Impact of drip fertigation on productivity of arecanut (Areca catechu L.). Agricultural Water Management 90, 101–111.

Bremner J.M., 1965. Method of Soil Analysis. Part 2. Chemical and Microbiological Methods. American Society of Agronomy Inc. Madison, Wise USA., 1149-1178.

Castellanos M.T., Tarquis A.M., Ribas F., Cabello M.J., Arce A., Cartagena M.C., 2012. Nitrogen fertigation: an integrated agronomic and environmental study. Agricultural Water Management,http://dx.doi.org/10.1016/j.agwat.2012.06.016.

Doorenbos J., Kassam A.H., 1979. Yield response to water. Irrigation and drainage paper no: 33. FAO-Rome, 193.

Drew M.C., 1975. Comparison of the effects of localized supply and the growth of the seminal root system in barley. II. Compensatory increases in the growth of lateral roots, and in rates of phosphate uptake, in response to a localized supply of phosphate. J. Exp. Bot. 29:435-451.

Erdem Y., Yüksel A.N., Orta A.H., 2001. The effects of deficit irrigation on watermelon yield, water use, and quality characteristics. Pak J. Biol Sci 4(7):785–789.

Fernandes F.M., Prado R.M., 2004. Fertirrigação da cultura da melancia. In: Boaretto, A.E.; Villas Boas, R.; Souza, W.F.; Parra, L.R.V. (Eds.) Fertirrigação: teoria e prática. Piracicaba, vol.1, 632-653.

Grangeiro L.C, Cecílio Filho A.B., 2004. Acúmulo e exportação de macronutrientes pelo híbrido de melancia Tide. Hortic. Bras., 22, 93-97.

Gündüz M., Kara C., Bilgel L., Değirmenci V., 1996. Determination of irrigation scheduling of watermelon in Harran plain. In: Pakyurek A (ed) The first vegetable conference proceedings. Harran, Sanlıurfa-Turkey, pp 211–216.

Hagin J., Sneh M., ve Lowengart A., 2002. Fertigation – Fertilization through irrigation. IPI Research Topics No. 23. Ed. by A.E. Johnston. International Potash Institute, Basel, Switzerland.

Hochmuth G.J., 1992. Fertilizer management for drip-irrigated vegetables in Florida. HortTechnology. 2, 27-32.

Mahajan G., Singh K.G., 2006. Response of Greenhouse tomato to irrigation and fertigation. Agricultural Water Management 84, 202–206.

Miller G., 2002. Home and Garden Information Center (Excerpted from *Home Vegetable Gardening*, EC). County Extension Agent, Clemson University, ABD. http://hgic.clemson.edu/factsheets/hgic1325.htm.(*Erişim Tarihi: 30/06/2008*).

Mohammad M.J., ve Zuraiqi S., 2003. Enhancement of yield and nitrogen and water use efficiencies by nitrogen drip-fertigation of garlic. Journal of Plant Nutrition. 26 (9), 1749-1766.

Murphy L., Riley J. P., 1962. A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. Anal. Chem. Acta. 27:31-36.

Riley D., ve Barber A., 1971. Effect of ammonium fertilization on phosphorus uptake as related to root-induced pH changes at the root-soil interface. Soil Sci. Soc. Am. Proc. 25: 301-306.

Silva M.V.T. da, Chaves S.W.P., Medeiros J.F., de Souza M.S., de Santos A.P.F., 2012. Acúmulo e exportação de macronutrientes em melancieiras fertirrigadas sob ótimas condições de adubação nitrogenada e fosfatada. Agropecuária Científica no Semi-Árido 8, 61-70.

Simsek M, Kacira M, Tonkaz. T., 2004. The effects of different drip irrigation regimes on watermelon yield and yield components under semi-arid climatic conditions. Aust J Agric Res 55:1149–1157.

Soon, Y.K., ve Miller, H., 1977. Changes in rhizosphere due to ammonium and nitrate fertilization and phosphorus uptake by corn seedlings. Soil Sci. Soc. Am. Proc. 41:77-80.

Srinivas K., Hegde D.M., Havanagi C.V., 1989. Irrigation studies on watermelon. Irrigation Sci 10 (4):293–301.

Taskaya B., Keskin G., 2004. Kavun-karpuz. Tarımsal Ekonomi Araştırma Enstitüsü, Sayı 6, Ankara.

Vidigal S.M., Pacheco D.D., Costa E.L., da Facion C.E., 2009. Crescimento e acúmulo de macro e micronutrientes pela melancia em solo arenoso. Rev. Ceres. 56, 112-118.

Yetişir H., Sari N., 2004. Effect of Hypocotyl Morphology on Survival Rate and Growth of Watermelon Seedlings Grafted on Rootstocks with Different Emergence Performance at Various Temperatures. Turk. J. Agric., 28, 231-237.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ESTABLISHING THE CROP ASSORTMENT OF WATER MELON (*CITRULLUS LANATUS)* DEPENDING ON THE ELEMENTS THAT DEFINE THE PRODUCTION

Mihaela Gabriela CIUPUREANU (NOVAC)[2], Elena CIUCIUC[1], Daniela POPA[2]

[1]Research - Development Center For Field Crops on Sandy Soils Dăbuleni, Romania
[2]University of Craiova, Faculty of Horticulture, Doctoral School of Engineering Plant and Animal Resources, 13 A.I. Cuza Street, Craiova, Dolj, Romania

Corresponding author email: danapopa2013@gmail.com

Abstract

The aim of this study it was that of following the behavior in culture on field on RDCFCSS Dabuleni – Romania, on a sandy soil, of 12 cultivars of water melons of which 9 are hybrids of foreign provenance and 3 local varieties. It were made observations and determinations concerning the morphological characters and the production of the cultivars such as: the vegetative growth of plants, the flowering and the fruits binding date, the number of fruits/plants, the average weight of a fruit and the productions on cropping stages. Following the obtained results it was found that the highest production was registered on 'Baronesa' F1 cultivars and 62-269 F1 with 47.9 t/ha, followed by LF 6720 with 44.9 t/ha and 'Oltenia' with 43.6 t/ha.

Key words: *water melon, temperature, fruits, production.*

INTRODUCTION

Nowadays, in a competitive market conditions, the cultivators of vegetable species are becoming more interested to cultivate varieties and hybrids of foreign provenance with an acclimatization risk to the natural conditions of the specific area.
The choice of varieties and hybrids in relation to pedo-climatic conditions of the area it is possible only after a preliminary testing of those. Because the water melon fruits are consumed only in fresh conditions, the gustative qualities of those one have a particular importance.
The normal progress of the metabolic processes on vegetable plants is closely related to environmental factors. The direct factors that acts directly on plants, representing their condition of existence, are: climatic factors (light, heat, water and air) edaphic factors (the texture and the soil structure the chemistry and the trophicity of soil, the groundwater) and biotic factors (human and other living organisms) (Voican et al., 1998). From the point of view of requirements for temperature, the sandy soils area from the South of Oltenia can be considered a very favorable area for growing water melons (Toma et al., 2007).
In the last decades the humanity is facing more and more with the effects of global change, customized on regional level. The increase of water melons productions is based on the use of biological material variety, hybrid) that are becoming more performant, that through the genetic heritage that they possessed it can adapt to the environmental conditions of the area.
Choosing the best varieties and hybrids in relation to the customer requirements and to the pedo-climatic conditions from the area, is possible only knowing in detail the specific of as many varieties and hybrids of water melons existing in our country and abroad (Maria Dinu et al., 2016).
The water melons profitable capitalize the pedo- climatic conditions specific to the sandy soils under the application of an irrigation regime well led (Marinica, 1998). For the sandy soils area, Nanu (1998), recommend the cultivars: 'Dulce de Dăbuleni', 'Crimson Sweet' and 'Red Star' F1 hybrid. The assortment of yellow and green water melons periodically renewed due to the appearance of new creations and in accordance with the

market requirements. As a result, it is therefore necessary to study some cultivars (varieties and hybrids) with high adaptability to the eco-pedological specific conditions of the sandy soils in order to introduce them in culture.
The aim of this study was that to track the behavior in pedo-climatic conditions from the Southern Romania (RDCFCSS Dăbuleni) of 12 cultivars of water melon from which 3 varieties are aborigine creations and 9 foreign hybrids in order to recommend the most valuable for adaptability and productivity.

MATERIALS AND METHODS

The present study was conducted in the experimental field of RDCFCSS Dabuleni in 2016.
The experience was mono factorial and was placed after the method of randomized blocks. It has 12 variants with 4 repetitions and consists in 3 varieties and 9 hybrids.
The surface of a variant was of 20 m^2. The cultural experimental scheme was of 200 x 100 cm.
The variants specific were:
V1 – 'De Dăbuleni',
V2 – 'Dulce de Dăbuleni',
V3 – 'Oltenia',
V4 – 'Susy' F1,
V5 – 'Baronesa' F1,
V6 – 'Oneida' F1,
V7 – 'Huelva' F1,
V8 – '62-269' F1,
V9 – 'Fantasy' F1,
V10 – 'Tarzan' F1,
V11 – 'Grand Baby' F1,
V12 – 'LF 6720' F1.
The experience was established by planting the seedling on 28 April 2016, previously produced in a solar greenhouse. The maintenance works applied to the culture it was the general specific ones to a water melon crop. Regarding the crop particularities, they relate to the use of plastic for mulch and to the use of drip irrigation system. It was performed morphological determinations on plants that were focused on the haulm length and also production determinations (number of fruits/plants, the average weight of a fruit, the total production). The obtained results were calculated and interpreted statistically.

RESULTS AND DISCUSSIONS

Regarding the climatic conditions in which was placed the experience (in field, on a sandy soil) it can say that the year 2016 (table 1)it has been especially from climatic point of view, excepting May month, in all the other months have recorded average temperatures over of each month multi annual average temperature.

Table 1.The climatic conditions - April - July 2016

Month	Specification	Temperature (^{0}C)	Rainfalls (mm)
April	Minimum	0.8	60.2
	Maximum	31.4	
	Monthly average	**15.0**	
	Multiannual average	12.1	
May	Minimum	5.5	104.4
	Maximum	32.9	
	Monthly average	**16.9**	
	Multiannual average	17.4	
June	Minimum	16.9	53.2
	Maximum	37.7	
	Monthly average	**23.6**	
	Multiannual average	21.4	
July	Minimum	11.4	31.6
	Maximum	38.0	
	Monthly average	**24.8**	
	Multiannual average	23.2	

April was particularly warm registering a maximum temperature of 31.4^{0}C and a medium temperature of 15^0 C much higher from the multiannual average temperature of the month that it is of 12.1^{0}C.
The amount of precipitations from April was of 60.2 mm. The May was rich in rainfalls for the area, registering 104.4mm leading to a decrease of temperatures.
The minimum temperature of the month was of 5.5^{0}C which negatively influenced the plants of water melons hardly planted.
The maximum of the month was of 32.9^{0}C. The May monthly average was of 16.9^{0}C lower than the multiannual average of the month with 0.5^{0}C.

June and July were very warm and poor in rainfalls, the minimum temperatures exceeding the multiannual average. Thus, in June it was registered in average 23.6^{0}C compared to 21.4^{0}C which is the multiannual average with a minimum temperature of 16.9^{0}C and a maximum temperature of 37.7^{0}C, and the amount of precipitations were of 53.2 mm.

The medium temperature of July was of 24.8^{0}C with limits between 11.4 – 38.0^{0}C on the background of some low rainfalls, respectively 31.6 mm.

During the vegetation period it were made observations regarding the dynamic of the vegetative growth of the plants on each cultivar in part (Table 2).

The length of the water melons haulm on the fruit bind time it was between 1.84-2.35 m. It have been note by vigor the cultivars: ‘Huelva’ F1 (2.35 m), ‘Oneida’ F1 (2.14 m), ‘Dulce de Dăbuleni’ (2.11 m), ‘LF 6720’ F1 (2.06 m) and ‘Oltenia’ (2.03 m).

The phenological stage of water melon plant flowering highlights ‘Oneida’ and ‘Huelva’ cultivars (02.06.2016) then ‘Grand Baby’ and ‘LF 6720’ (03.06.2016) and then the Romanian varieties that have a deferall against the foreign hybrids of approximately 10 days. The set of the first fruits occured in the period of 9 - 15 June on cultivars of foreign origin and in the period of 20 - 24 June on indigenous cultivars.

This difference of fruit setting between Romanian and foreign cultivars is unfavorable to the sandy area in which the water melon crops are set up mainly for the production precociously.

Table 2. Morphological and production determinations at the cultivars taken into study (minimum values)

Cultivar	Length of haulm (m)	Flowering time	Time of binding - first fruits
‘De Dăbuleni	1.86	13.06	22.06
‘Dulce de Dăbuleni’	2.11	10.06	24.06
‘Oltenia’	2.03	10.06	20.06
‘Susy’ F1	1.91	2.06	9.06
‘Baronesa F1	1.98	6.06	13.06
‘Oneida’ F1	2.14	2.06	10.06
‘Huelva’ F1	2.35	2.06	10.06
‘62-269’ F1	1.88	7.06	15.06
‘Fantasy’ F1	1.84	6.06	13.06
‘Tarzan’ F1	1.89	7.06	15.06
‘Grand Baby’ F1	1.86	3.06	10.06
‘LF 6720’ F1	2.06	3.06	10.06

The cropping was done at technological maturity, and the production was registered on each variant in part, on harvesting stages. The first harvest (table 3) was on 13 July on foreign cultivars and on 22 July on indigenous cultivars

Table 3. The dynamic of water melon production depending on cultivar (average values)

Cultivar	Producțion (t/ha) on:		
	13 July	22 July	1 August
‘De Dăbuleni’	-	25.0	8.8
‘Dulce de Dăbuleni’	-	23.1	12.2
‘Oltenia’	-	21.0	22.6
‘Susy’ F1	13.3	17.5	1.8
‘Baronesa’ F1	13.9	28.5	5.5
‘Oneida’ F1	14.6	15.6	8.2
‘Huelva’ F1	18.0	18.8	5.0
‘62-269’ F1	19.0	25.0	3.9
‘Fantasy’ F1	8.8	24.1	-
‘Tarzan’ F1	8.2	22.0	-
‘Grand Baby’ F1	16.3	25.3	-
‘LF 6720’ F1	16.4	28.5	-

The productions obtained on 13 July were between 8.2 t/ha at ‘Tarzan’ F1 cultivar and 19 t/ha at ‘62-269’ F1 cultivar. It also noted through the registered production on this date the cultivars ‘Huelva ‘F1 with 18 t/ha, ‘Grand Baby’ F1 with 16,3 t/ha and ‘LF 6720’ F1 with 16,4 t/ha.

At the second stage of harvest (22 July) all productions increased at all cultivars being between 15.6-28.5 t/ha. The cultivars ‘Fantasy’ F1, ‘Tarzan’ F1, ‘Grand Baby’ F1 and ‘LF 6720’ F1 completed their period of vegetation on 22 July, and the other cultivars on 1st of August.

At the last stage of harvesting, respectively at 1st of August the productions yield were between 1.8 t/ha at ‘Susy’ F1cultivar and 22.6 t/ha at ‘Oltenia’ cultivar. The average number of fruits/plants harvested was between 1.5-2 fruits/plants (table 4). The lowest number of fruits per plant was recorded at ‘Fantasy’ F1 cultivar, while the highest one was recorded at cultivars ‘Oneida’ F1, ‘Huelva’ F1 and ‘LF 6720’ F1. A healthy plant of water melon can produce 1-4 fruits per harvest.

Regarding the average weight of a fruit of water melon this ranged between 3.6-5.3 kg/fruit. The biggest fruits were recorded on cultivars ‘Oltenia’, ‘Baronesa’ F1 and ‘62-269’ F1. These registered results are confirmed by the existent data from literature. A study conducted by Sari et al. (2007) present results according to the average weight of water melon fruits ranged between 1.885-8.033 kg. The similar results were reported also by Pakyurek şi Yanmaz (2008), in a study of a genotypes assortment of 13 water melons, where it was identified an average fruits weight between 1 – 4 kg. Ayhan et al. (2014) identified some cultivars of water melon with an average weight of fruits between 1.29-3.96 kg. Cordova et al. (1995) classified the water melons according to the weight of fruits in this way: the fruits with a weight of 4 kg are considered as being small, the fruits with a weight between 4 - 6 kg are considered as being medium and the fruits with a weight between 8 - 12 kg are considered as being giant.

Table 4. The number of fruits per plant and the average weight water melon fruits depending on cultivar

Cultivar	Number of fruits/plant	Fruit weight (kg)
'De Dăbuleni'	1.8	3.8
'Dulce de Dăbuleni'	1.8	3.8
'Oltenia'	1.6	5.3
'Susy' F1	1.7	3.6
'Baronesa' F1	1.9	5.0
'Oneida' F1	2.0	4.0
'Huelva' F1	2.0	4.0
'62-269' F1	1.8	5.2
'Fantasy' F1	1.5	4.4
'Tarzan' F1	1.6	3.9
'Grand Baby' F1	1.9	4.4
'LF 6720' F1	2.0	4.5

The production of water melon (table 5) registered at the 12 cultivars taken into study was between 32.6 – 47.9 t/ha.
The biggest productions as against the control were achieved on cultivars 'Baronesa' F1 and '62-269' F1 which realized 47,9 t/ha, followed by 'LF 6720' with 44.9 t/ha 'Oltenia' with 43.6 t/ha, 'Huelva' F1 with 41.8 t/ha and 'Grand Baby' F1 with 41.6 t/ha. It was elected the cultivar 'Oltenia' as control because it is cultivated mostly in Dabuleni area and it produce appreciable quantities of fruits. The production differences as against the control were between 4.3-1.3 t/ha, having positive differences. The smallest productions of water melon were obtained at cultivars 'Susy' F1, 'Fantasy' F1 and 'De Dăbuleni'.

Table 5.The total production at the water melon studied cultivars (average values)

Cultivar	The obtainded production (t/ha)	± Dif. As against Mt.(t/ha)
'De Dăbuleni'	33.8	-9.8
'Dulce de Dăbuleni'	35.3	-8.3
'Oltenia'	43.6	Mt.
'Susy' F1	32.6	-11.0
'Baronesa' F1	47.9	+4.3
'Oneida' F1	38.4	-5.2
'Huelva' F1	41.8	-1.8
'62-269' F1	47.9	+4.3
'Fantasy' F1	32.9	-10.7
'Tarzan' F1	30.2	-13.4
'Grand Baby' F1	41.6	-2.0
'LF 6720' F1	44.9	+1.3

DL 5% = 17.30 t/ha;
DL 1% = 23.57 t/ha;
Dl 0.1% = 31.68 t/ha.

The production of 'Oltenia' variety is remarkable for the sandy area from the Southern Romania, being the only Romanian variety with a production over 40 t/ha.
Between the foreign cultivars only five of them registered productions over 40 t/ha, three of them being precocious (62-269 F1, 'Huelva' F1 and 'LF 6720' F1).

CONCLUSIONS

From the obtained results about the behaviour in culture, in the pedoclimatic conditions from RDCFCSS Dăbuleni we notice the following cultivars:
- concerning the number of fruits/plant, 'Oneida' F1, 'Huelva' F1 and 'LF 6720' F1, and by the average weight of a water melon fruit, the cultivars 'Oltenia', 'Baronesa' F1 and '62-269' F1;
- precociousness was noted by the cultivars '62-269' F1 with 19 t/ha and 'Huelva' F1 with 18 t/ha on 13th of July.
- the biggest production was recorded on cultivars 'Baronesa' F1 and '62-269' F1 that achieved 47.9 t/ha, followed by 'LF 6720' with 44.9 t/ha; 'Oltenia' with 43.6 t/ha; 'Huelva' F1 with 41,8 t/ha and 'Grand Baby' F1 with 41.6 t/ha.

ACKNOWLEDGMENTS

The results were part of a research made in collaboration between the University of Craiova and the Research-Development Center For Field Crops On Sandy Soils Dăbuleni, for a doctoral thesis.

REFERENCES

Ayhan G., Nebahat S., Ilknur S., 2014. See Yield and Quality of Watermelon Genotypes Having Snack Food Potential. Cucurbitaceae Proceedings, Michigan, USA, 57- 62.

De Cordova F., Diez M.J., Iglesias A., Nuez F., 1995. Germplasm resources of *Citrullus lanatus* in the genebank of the Polytechnic University of Valencia, Cucurbit Genetics Cooperative 28:52–54.239

Dinu M., Soare R., 2016.The influence of cultivar on the quality of fruit the species *Cucumis melo* L. Annals of the University of Craiova, Agriculture, Montanology, Cadastre Series. Vol.XLVI, No 2, 105-111.

Marinică Gh., 1998. Cercetări privind regimul de irigare aplicat pepenilor verzi pe solurile nisipoase amenajate din sudul Olteniei. Lucrări științifice SCDCPN Dăbuleni, vol. X, 161-167.

Nanu Șt., 1998. Soiuri și hibrizi de pepeni verzi cultivațe pe solurile nisipoase din sudul Olteniei *Citrullus lanatus* (Thunb.) Matsum et Nakai. Lucrări științifice SCDCPN Dăbuleni, vol. X, 124-130.

Nanu Șt., Toma V., 2003. Soiuri și hibrizi de pepeni galbeni cultivați pe solurile nisipoase din sudul Olteniei. Lucrări științifice SCDCPN Dăbuleni, Volumul XV, Ed. SITECH Craiova, 159-164.

Pakyurek A.Y., Yanmaz R., 2008. Çerezlik karpuz (*Citrullus lanatus* (Thumb.) Matsum.) yetistiriciligine uygun gen kaynaklarının toplanması ve degerlendirilmesi uzerine arastırmalar. VII th Symposium of Vegetable Crops, Yalova, 236.

Sari N., Aka Kacar Y., Yalcin Mendi Y., Solmaz I., Aktas H., 2007. Morphological and genetic characterization of watermelon genetic resources. TUBITAK Project No:104O073.

Toma V., Ciuciuc Elena, Croitoru Mihaela, Ploae Marieta, 2007. Comportarea unor cultivare de pepeni verzi în cultură altoită pe solurile nisipoase din sudul Olteniei. Lucrări științifice SCDCPN Dăbuleni, vol. XVI., 129-139.

Voican V., Lăcătuș V., 1998. Cultura protejată a legumelor în sere și solarii. Editura CERES, București. ISBN 973-40-0398-4.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

GOOD AGRICULTURAL PRACTICES (GAP) FOR GREENHOUSE SOILLESS TOMATO GROWING

Nilda ERSOY[1], Osman TEKİNARSLAN[2], Ulaş GÖKTAŞ[3], Elif AKÇAY ÖZGÜR[2], Ayşe METİN[4], Ramazan GÖKTÜRK[5], Gürkan KAYA[6]

[1]Akdeniz University, Vocational School of Technical Sciences, Program of Organic Agriculture, 07058, Antalya, Turkey
[2]ECAS Inspection and Certification Organization, 07075, Antalya, Turkey
[3]PROANALİZ Laboratories Group, Antalya, Turkey
[4]ESAY Agriculture Consulting, 07310, Antalya, Turkey
[5]Akdeniz University, Faculty of Science, Department of Biology, 07058, Antalya, Turkey
[6]Mehmet Akif Ersoy University, Faculty of Engineering and Architecture, 15030, Burdur, Turkey

Corresponding author email: nildaersoy@akdeniz.edu.tr

Abstract

Tomatoes have become one of the most popular and widely grown vegetables around the world. Nowadays, tomato cultivation with soilless agriculture has gained popularity. Sustainability of good farming practices seems to be quite high compared to other agricultural production systems. This research was carried out in the Serik District of Antalya to demonstrate the effectiveness of good agriculture practices in the greenhouse cultivation of soilless tomatoes. The varieties taken in the experiment were Gülpembe grafted on Beaufort rootstock. In the study, pesticide residue levels were determined in fruit extracts as well as analyses for water. Extraction steps and all analyses were carried out at the Proanaliz Food Control Laboratory. High-precision analytical instruments such as LC-MS / MS and GC-MS were used for pesticides residues. A total of 1025 pesticide active substances were analysed in LC-MS / MS and 117 pesticide active substances in GC-MS in fruit extracts. In this research carried out in 2015 and 2016, samples of both years were not found to be detectable to the tolerance values of Turkish Food Codex (TFC).

***Key words:** tomatoes, GAP, pesticides, residue, Serik-Antalya.*

INTRODUCTION

Tomato is one of the most important vegetables produced in the world. Its homeland is the South America Countries where Peru and Ecuador are located. It was first cultivated by Mexicans and after the discovery of New World, it spread from America to Europe and the other continents. In Turkey, it was started to be grown in the early 1900s in Adana.
On the species basis, the tomato that takes the first place in vegetable cultivation is cultivated both open field and in greenhouses.
In Turkey 12,615,000 tons of tomatoes are produced, of which 8,170,000 tons are table tomatoes, 4,445,000 tons are for sauce production (TÜİK, 2016).
Greenhouse production has an important place in our agricultural sector due to its high efficiency and income from the unit side and ,at the same time, providing a regular use of labor force throughout the year by spreading vegetable production to all seasons of the year. In greenhouse production, tomato takes the first place.
Approximately 27% (3,399,100 tons) of our total tomato production is obtained from the greenhouse and contribution of this specie in greenhouse production is 53.5%. 77.6% of greenhouse tomato production is originating from the greenhouse systems in the Mediterranean Region.
Antalya provides 62.5% of this production. Greenhouse tomato production is practiced in 259,709 da areas. 5.6% of production area consists of low plastic tunnel (14,644 da), 6.1% high plastic tunnel (15,765 da), 68.5% plastic greenhouse (177,937 da) and 19.8% glass greenhouse areas (51,363 da). Most of the production is obtained from plastic greenhouses (71%) and glasshouses (21%).
The amount of greenhouse tomato production in Turkey has increased prominently in recent

years due to increase of production area, usage of quality seed and modern agricultural techniques. (TÜİK, 2016).
Soilless production in Turkey besides having a history of about 20 years, is rapidly becoming widespread due to providing earliness, productivity and quality increase.
Soilless agriculture is generally widespread in the Mediterranean and Aegean Regions and 74% of total production is made in Antalya and İzmir provinces.
The most important specie in soilless agriculture is tomato (Toprak and Gül, 2013; Kandemir et al., 2015).
Soilless agriculture is based on the principle that water and nutrients required for plant growth are supplied to the root in the required amount and it is split up into environmental and aquatic culture.
In environmental culture, plants are grown in organic or inorganic environments with irrigation nutrition solution. In aquatic culture, plants are grown in water containing nutrients.
Food safety and quality are among the most important issues in recent years.
The residues of the pesticides have become the fear of those who wants healthy foods. The consumption of pesticides in the Mediterranean and Aegean regions, where greenhouses are common, is close to two-thirds of the country's total.
On the other hand, when we think features of pesticides we consume, the vast majority have significant risks for human and environmental health.
Residue analyzes which are made more frequently than the past show that pesticide contamination in our products is reduced, but even in our elite products exported to EU countries, we encounter parcels which are not suitable for pesticide residue limits.
Besides all these problems, a number of new legal regulations to solve problems caused by pesticides, the prohibition of some risky pesticides, the introduction of a prescription system, and some other planned regulatory developments are seen as promising developments (Anonymous, 2017a).
Another of these promising developments, which has a great importance, is undoubtedly good agricultural practices based on certificated production. Antalya has an important position in vegetable, especially tomato growing. In such places where the potential is high, it is necessary to speed up the works for good agricultural practices.
In this research, it is aimed to determine pesticide residues obtained from a greenhouse, in which are grown tomatoes with good agricultural practices, in hydroponic system in Antalya.

MATERIALS AND METHODS

Materials

Materials in this research are varieties of truss tomato called 'Gülpembe F1' grafted onto rootstock. This kind of plant is in the category of pitted tomato, the shortness of internode is short, early and highly productive. There are 4-5 fruits in the bunch, they are small sliced, their taste and aroma are so good, colour is bright pink, weight 250 g, flesh is hard and its shelf life is ideal.
Pesticides given in Table 1 and Table 2 are searched in the examples of tomatoes, which are the materials. All extraction studies and residue analysis of the examples made in Proanaliz Food Control Laboratory.

Table 1.Active substances examined in tomatoes examples on LC-MS/MS device

2,4,5-T; 2,4-D; 3,4,5 trimethacarb; Abamectin; Acephate; Acequinocyl; Acetamiprid; Acetochlor; Acibenzolar-S-Methyl; Aclonifen; Acrinathrin; Alachlor; Aldicarb; Aldicarb-Sulfone; Aldicarb-Sulfoxide; Allethrin; Ametoctradin; Ametryn; Amidosulfuron; Amisulbrom; Amitraz; Amitrole; Anilazine; Anilofos; Aramite; Asulam; Atrazine; Azamethiphos; Azimsulfuron; Azinphos-Ethyl; Azinphos-Methyl; Aziprotryne; Azoconazole; Azocyclotin; Azoxystrobin; Barban; Beflubutamid; Benalaxyl; Bendiocarb; Benfuracarb; Benomyl; Bensulfuron methyl; Bentazone; Benthiovalicarb Isopropyl; Benzoximate; Bifenox; Bifentrin; Binapacryl; Bioresmethrin; Bispyribac; Bitertanol; Boscalid; Bromacil; Bromophos methyl; Bromophos-Ethyl; Bromoxynl; Bromuconazole; Bupirimate; Buprofezine; Butafenacil; Butocarboxim; Butocarboxim-sulfone; Butocarboxim-sulfoxide; Butoxycarboxim; Butralin; Buturon; Butylate; Cadusafos; Campheclor; Campheclor-methyl; Campheclor-oxon; Campheclor-oxon-sulfone; Campheclor-oxon-sulfoxide; Campheclor-sulfone; Campheclor-sulfoxide; Carbaryl; Carbendazim; Carbofuran; Carbosulfan; Carboxin; Carfentrazone-Ethyl; Chlorantraniliprole; Chlorbromuron; Chlorbufam; Chlorfenvinphos; Chlorfluazuron; Chloridazon; Chlormequat chloride; Chlorotoluron; Chloroxuron; Chlorpropham; Chlorpyrifos; Chlorpyrifos-Methyl;

Chlorsulfuron; Chlortal-dimethyl; Chlorthiamid; Chromafenozide, Cinidon-ethyl; Clethodim; Clethodim Iminsulfone; Clethodim Iminsulfoxide; Clethodim Sulfoxide; Climbazole; Clodinafop-proparpyl ester; Clofentezine; Clomazone; Cloquintocet-methylhexyl ester; Clothianidin; Coumaphos; Crimidine; Cyanazine; Cyanofenphos; Cyazofamid; Cyclanilide; Cycloate; Cycloloxydim; Cyflufenamid; Cyhalofop; Cyhalofop butyl; Cyhalofop diacid; Cyhexatin; Cymoxanil; Cyproconazole; Cyprodinil; Cyromazine; Daminozide; Dazomet; Demeton(O+S); Demeton-S-Methyl; Demeton-S-Methyl-Sulfone; Demeton-S-Methyl-Sulfoxide; Desmedipham; Desmetryn; Diafenthiuron; Dialifos; Di-Allate; Diazinon; Dichlofenthion; Dichlofluanid; Dichlorprop; Dichlorvos (DDVP); Diclobutrazol; Diclofop-Methyl; Dicloran; Dicrotophos; Diethofencarb; Difenoconazole; Diflubenzuron; Diflufenican; Dimefox; Dimethachlor; Dimethenamid; Dimethoate; Dimethomorph; Dimetilan; Dimoxystrobin; Diniconazole; Dinitramine; Dinocap; Dinoseb; Dinoterb; Dioxacarb; Diphenamid; Dipropetryn; Disulfoton; Disulfoton Sulfone; Disulfoton Sulfoxide; Ditalimfos; Dithianon; Diuron; DNOC; Dodine; E-Fenpyroxymate; Emamectin; Benzoate; Endosulfan Sulfate; Epichlorohydrin; EPN; Epoxiconazole; EPTC; Esfenvalerate; Etaconazole; Ethalfluralin; Ethametsulfuron Methyl; Ethiofencarb; Ethiofencarb-sulfone; Ethiofencarb-sulfoxide; Ethion; Ethiprole; Ethirimol; Ethofenprox; Ethofumesate; Ethoprophos; Ethoxyquin; Ethoxysulfuron; Ethylene thiourea; Etoxazole, Etridiazole; Etrimfos; Famoxadone; Famphur, Fenamidone; Fenamiphos; Fenarimol; Fenazaquin; Fenbuconazole; Fenbutatin oxide; Fenhexamid; Fenitrothion; Fenobucarb; Fenoxyaprop-P-ethyl; Fenoxycarb; Fenpiclonil; Fenpropathrin; Fenpropidin; Fenpropimorph; Fensulfothion; Fenthion; Fenthion Oxon; Fenthion Oxon Sulfone; Fenthion Oxon Sulfoxide; Fenthion-Sulfone; Fenthion-Sulfoxide; Fentin acetate; Fentin Hydroxide; Fenvalerate; Fipronil; Flamprop-M-Isopropyl; Flazasulfuron; Flonicamid; Florasulam; Fluazifop-p-butyl; Fluazinam; Flubendiamide; Flubenzimine; Flucycloxuron; Flucythrinate; Fludioxonil; Flufenacet; Flufenoxuron; Flumioxazine; Fluometuron; Fluopicolide; Fluopyram; Fluorochloridone; Fluoroglycofen Ethyl; Fluoxastrobin; Flupyrsulfuron Methyl; Fluquinconazole; Fluroxypyr; Flurtamone; Flusilazole; Flutolanil; Fluxapyroxad; Fomesafen; Fonofos; Foramsulfuron; Forchlorfenuron; Formetanate; Formetanate hydrochloride; Fosthiazate; Fuberidazole; Furalaxyl; Furathiocarb; Halfenprox; Halosulfuron Methyl; Haloxyfop-2-Ethoxy-Ethyl; Heptanafos; Hexaconazole; Hexaflumuron; Hexazinone; Hexythiazox; Imazalil; Imazamox; Imazapic; Imazapyr; Imazaquin; Imazethapyr; Imazosulfuron, Imibenconazole; Imidachloprid; Indoxacarb; Iodosulfuron methyl sodium; Ioxynil; Ipconazole; Iprobenfos; Iprodione; Iprovalicarb; Isazofos; Isocarbofos; Isoprocarb; Isoproturon; Isoxaben; Isoxadifen Ethyl; Isoxaflutole; Isoxathion; Kinetin; Kresoxim-methyl; Lenacil; Linuron; Lufenuron; Malaoxon; Malathion; Maleic Hydrazide; Mandipropamide; MCPA; Mecarbam; Mecoprop (MCPP); Mecoprop-P (MCPP-P); Mepanipyrim; Mephosfolan; Mepronil; Meptyldinocap; Mesosulfuron Methyl; Mesotrione; Metaflumizone; Metalaxyl; Metalaxyl M; Metamitron; Metazachlor; Metconazole; Methabenzthiazuron; Methacrifos; Methamidophos; Methidathion; Methiocarb; Methiocarb sulfone; Methiocarb sulfoxide; Methomyl; Methomyl oxime; Methomyl Sulfone; Methoxyfenozide; Metobromuron; Metolachlor; Metolcarb; Metosulam; Metoxuron; Metribuzin; Metrofenone; Metsulfuron-Methyl; Mevinphos; Milbemectin A3; Milbemectin A4; Molinate; Monocrotophos; Monolinuron; Monuron; Myclobutanil; Naled (Dibrom); Naphthalene Acetamide (NAD); Napropamide; Napthol-1; Neburon; Nicosulfuron; Nitenpyram; Norfluazuron; Novaluron; Nuarimol; Ofurace; Omethoate; Orthosulfamuron; Oxadiazon; Oxadixyl; Oxamyl; Oxasulfuron; Oxycarboxin; Oxyfluorfen; Paclobutrazol; Paraoxon Ethyl; Paraoxon Methyl; Parathion-Ethyl; Parathion-Methyl; Pebulate; Penconazole; Pencycuron; Pendimethalin; Penoxsulam; Permethrin; Pethoxamid; Phenmedipham; Phenothrin; Phentoate; Phorate; Phorate Sulfone; Phosalone; Phosmet; Phosmet oxon; Phosphamidon; Phoxim; Picolinafen; Picoxystrobin; Pinoxaden; Pirimicarb; Pirimicarb Desmethyl; Pirimicarb Desmethyl Formamido; Pirimiphos-Ethyl; Pirimiphos-Methyl; Prochloraz; Profenofos; Profoxydim; Profoxydim lithium; Prohexadione calcium; Promecarb; Promethryn; Propachlor; Propanil; Propaquizafop; Propargite; Propazine; Propetamphos; Propham; Propiconazole; Propisochlor; Propoxur; Propoxycarbazone sodium; Propyzamide; Proquinazid; Prosulfocarb; Prosulfuron; Prothioconazole; Prothiophos; Pymetrozine; Pyraclostrobin; Pyraflufen; Pyraflufen ethyl; Pyrasulfotole; Pyrazophos; Pyrethrins; Pyridaben; Pyridaly; Pyridaphenthion; Pyridate; Pyrifenox; Pyrimethanil; Pyriproxyfen; Quaizalofop_P_Ethyl; Quinalphos; Quinclorac; Quinmerac; Quinoxyfen; Resmethrin; Rimsulfuron; Rotenone; Sethoxydim; Silthiofam; Simazine; Spinetoram; Spinosad; Spirodiclofen; Spiromesifen; Spirotetramat; Spirotetramat-Enol; Spirotetramat-Enol-Glucoside; Spirotetramat-Ketohydroxy; Spirotetramat-Monohydroxy; Spiroxamine; Sulcotrione; Sulfosulfuron, Sulfotep; Sulprofos; Tebuconazole; Tebufenozide; Tebufenpyrad; Tebupirimfos; Teflubenzuron; Tembotrione; Temephos; TEPP(O.O-TEPP); Tepraloxydim; Terbufos; Terbumeton; Terbuthylazine; Terbutryn; Tetraconazole; Tetramethrin; Thiabendazole; Thiacloprid; Thiamethoxam; Thidiazuron; Thifensulfuron-methyl; Thiobencarb; Thiodicarb; Thiofanox; Thiofanox Sulfone; Thiofanox Sulfoxide; Thiophanate-methyl; Tolclofos-Methyl; Tolfenpyrad; Topramezone; Tralkoxydim; Triadimefon; Tri-allate; Triasulfuron; Triazophos; Tribenuron-Methyl; Trichlorfon; Trichloronat; Triclopyr; Tricyclazole; Tridemorph; Triethyl Phosphate; Trifloxystrobin; Triflumizole; Triflumuron; Triflusulfuron Methyl; Triforine; Trinexapac Ethyl; Triticonazole; Tritosulfuron; Uniconazole; Vamidothion; Zoxamide

Table 2.Active substances examined in tomatoes examples on GC-MSD device

2,4-5T; 4,4 Dichlorobenzophenone; Aldrin (HHDN); Alpha-Endosulfan; Alphamethrin (cypermethrin); Beta-Endosulfan; Bromopropylate; Captafol; Captan; Chlorfenson; Chlorobenzilate; Chlorothalonil; Cycloate; Cyfluthrin; Cypermethrin; Dazomet; DDD-2.4'-; DDD-4.4'-; DDE-2.4'-; DDE-4.4'-; DDT-2.4'-; DDT-4.4'-; Deltamethrin; Dicamba; Dicofol; Dinobuton; Diphenylamine; Endosulfan-sulfate; Endrin; Esfenvalerate; Ethalfluralin; Fenvelarate; Folpet; Formothion; Gamma-HCH (Lindane); Hexachlorobenzene; Iprodione; IS-TPP; Lambda-Cyhalothrin;

Methoxychlor; Nitrofen; Nitrothal-isopropyl; Permethrin; Phenmedipham; Procymidone; Quintozene (penta chloro nitro benzene); Qunomethionate; Tau-fluvalinate; Tecnazene; Tetradiphon; Tetrasul; Thiometon; Tolyfluanid; Trifluralin; Vinclozolin; α (alpha)- HCH; β(beta) – HCH; 2-chloraniline; 3-chloraniline; 4-chloraniline; Aminocarb; Diclobenil; Biphenyl; Propamocarb; Carbofuran -3-hydroxy; Nitrapyrin; Chloroneb; 2-phenyl phenol; Benfluralin; BHC; Dioxathion; Profluralin; Fluchloralin; Terbacil; HCH(delta); Tefluthrin; Bromociclen; Pentacholoaniline; Flurprimidol; Chlorzolinate; Transfluthrın; Fenchlorphos; Diphenylmercury; Dinoseb acetate; Heptachlor endo-epoxide(trans isomer); Heptachlor endo-epoxide(cis isomer); S-Metolachlor; Fenson; Isodrin; Iodofenphos; Isofenphos; Chlorbenside; Haloxyfop-R-methyl; Tetrachlorvinphos; Chlordane-cis-alpha; Flutriafol; Diethatyl-ethyl; Dieldrin; Perthane; Chlorfenapyr; Carbophenothion, Tributyl Phosphote; Chlordane-trans-gamma; IS-TPP; Bifenazate; Mefenpyr-diethyl; Leptophos; Heptachlor; Mirex; Dimethipin; Cyanaphos; Chlorthion; Methoprene; Chlordecone; Oxadiargly; Fluotrimazole; Lactofen

Methods

All the solvents and chemicals (water, acetonitrile, methanol, formic acid, acetic acid and ammonium formate) used as mobile phases in example extractions are chosen in accordance to a profound quality. Pesticide standards are prepared at least a 90% rate of purity. Extractions and clearance of the examples are generalized in accordance with AOAC (International Official Methods of Analysis) methods (Lehotay, 2007). Some chromatographic conditions for LC-MS /MS and GC-MS devices are given in Table 3 and Table 4.

Table 3. Chromatographic Working Conditions of LC-MS/MS

LC-MS/MS	Agilent 6420			
Mobile Phase A	5 mM Amonium Formate&Water + Acetonitrile			
Mobile Phase A	Pure methanol			
Column	Poroshell 120 SB-C18 (3.0 x 100 mm 2.7 Micron)			
Injection Volume	10 µl			
Flow Rate	0.6 ml/min			
MS Gas Temperature	300°C			
Sheat Gas Temperature	350°C			
The Column Oven	35°C			
Pump Gradient Program				
	Time	Mobile phase A %	Mobile phase B %	Flow rate ml/min
	0:00	80	20	0.6
	0:00	80	20	0.6
	0:20	80	20	0.6
	1:50	30	70	0.6
	6:00	5	95	0.6
	7:50	5	95	0.6
	7:60	80	20	0.6
	10:00	80	20	0.6

Table 4. Chromatographic Working Conditions of GC/MS

GC-MS	Agilent 5975
Carrier gases	Helium
Column	HP-5MS 30 m × 250 µm × 250 µm × 0.25 µm
Injection Volume	5 µl
Flow Rate	2.4 ml/min
Duration of Injection	18.5 min
MS Gas Temperature	300°C
Sheat Gas Temperature	350°C
The Column Oven	35°C
Inlet temperature program	

Start	Rate of increase (°C/min)	Temperature (°C)	Retention Time (RT) (min)
1	0	55	0.21
2	600	325	18.5

The Column Oven temperature program			
Start	Rate of increase (°C/min)	Temperature (°C)	Retention Time (RT) (min)
1	0	50	0
2	50	150	0
3	20	230	1
4	8	290	3
5	0	290	18.5

RESULTS AND DISCUSSIONS

Residue quantities obtained from the research were evaluated as average of 3 repetitions in each sample according to Turkish Food Codex (TFC) Regulation on Maximum Residue Limits of Pesticides (Turkish Official Gazette No 21.01.2011-27822; Notification No: 2011/2). The TFC residue limits of each pesticide sample are indicated separately in the tables presented. In residue limits determined by using high-precision analytical instruments such as GC-MS and LC-MS/MS, in tomatoes samples analyses of total 1025 pesticide active ingredients were made in LC-MS/MS instrument and 117 pesticide active ingredients in GC-MS instrument. In this research carried out between 2015 and 2016, detectable levels of the residues were not found in the samples of these two years.

The use of conscious and controlled pesticides in our agricultural production, especially in exported products, is very important to avoid the residual problem. The use of pesticides should be very conscious and controlled to ensure the safety of our country's people and to protect our environment and our foreign trade. In the EU and the US, priority should be given to low-risk or environmentally friendly pesticides that have the potential to affect the environment and health as little as possible. In Turkey, environmentally friendly pesticides are not given priority to licensing and supporting of their consumption (Tiryaki, 2016).

Not having pesticide residues of agricultural products is very important in domestic consumption and foreign trade. Because the communication is very fast. In Rapid Alert System for Food and Feed (RASFF) of the EU is notified of which are not suitable due to pesticide residues. The EU has published products and origins on the internet which are not suitable for residues in the EU products through the Rapid Alarm System (Anonymous, 2017b). The eligibility status of the products exported to EU countries is shown in Table 5.

As seen in Table 5, the number of lots that are not suitable for the standards of foods and feedstuffs sent to the EU from Turkey and it rose even further in 2015.

Turkey is in the second place among 146 countries from the point of the number of non-eligible parties. Also, in a research we carried out, 203 pesticide residues in tomato, pepper and aubergine vegetables samples collected from local markets and markets in Konya were analyzed.

Extraction of vegetable samples was carried out in the laboratories of Selçuk University Departments of Agronomy and Horticulture, residue analyzes were made in the laboratories of Ministry of Food, Agriculture and Livestock, İzmir Provincial Control Laboratory Directorate's Organic Farming Products and Residue Analysis Laboratory with LC-MS/MS and GC-MS devices. Findings obtained from the study show that oxamyl (Tolerance value of Turkish Food Codex (TFC); 10 μg/kg), which is totally forbidden to use in a tomato sample, has a value of about 7 times, two different pesticides (112 μg/kg Ethion and 75 μg/kg Triazophos) were found in a pepper sample , in another pepper case, it was determined that 120 μg/kg Benomyl was above the tolerance value of 100 μg/kg of TFC.

In the 10 aubergine samples taken for the experiment, it was determined that the level of oxamyl which is prohibited to use was about 11 times, which means, 107 μg/kg. Besides, Imidiacloprid (TFC tolerance value; 20 μg/kg) was found respectively at 49, 190 and 64 μg/kg in 3 different aubergines.

Table 5. 2013-2015 notifications by country of origin

Country	Year 2013	Year 2014	Year 2015
China	436	417	388
Turkey	226	200	282
India	257	199	276
Spain	185	169	159
France	120	104	120
Poland	164	131	118
Germany	95	135	117
Italy	105	89	117
Netherlands	103	114	94
Brazil	187	109	91
United States	102	164	87
Vietnam	76	124	85
Egypt	49	55	78
Thailand	88	90	71
Iran	21	54	61
Belgium	60	75	58
United Kingdom	55	50	56
Denmark	19	28	27
Sweden	45	7	25
Argentina	76	40	22

In the same way, Osman et al. (2010) also investigated 23 different pesticide residues from GC-MS in 160 local vegetables collected from 4 large supermarkets in Al-Qassim district of Saudi Arabia (2010). According to the results, in 89 of 160 samples were found pesticide residues, in 53 it was determined that obtained values was above the Maximum Residue Levels (MRL). In this research, pesticide residues was found in 17 of 30 vegetable samples.

The most common pesticides in vegetables were respectively Carbaryl, Biphenyl and Carbofuran.

Zengin and Karaca (2017), investigated on 249 different pesticides residue levels in tomatoes samples which taken from greenhouse in Uşak province in 2015-2016 growing seasons. According to the results, 63% of taken 60 tomato samples had no detectable residues. In 37% of tomato samples detected pesticide residue, none of this pesticides exceeded the maximum residue limits given in Turkish Food Codex. Imidacloprid was the most common pesticide among detected pesticides.

Pesticide residues obtained from 11 of 16 pumpkin samples, 7 of 12 carrot and green pepper samples, 6 of 11 cucumber samples, 5 of 12 eggplant samples, 7 of 11 spinach samples, 6 of 11 lettuce samples, 4 of 11 tomato samples were reported to be above MRL.

The highest pesticide residues were found respectively in lettuce (Ethiofencarb, 7.648 mg/kg), tomato (Tolclofos-methyl, 7.312 mg/kg), pumpkin (Chlropyrifos, 6.207 mg/kg), carrot (Heptanophos, 3.267 mg/kg), green pepper (Carbaryl, 2.228 mg/kg) and aubergine (Carbaryl, 1.917 mg/kg). These findings point that it's necesssary pesticide residues in vegetables grown in greenhouses to be examined for the protection of public health.

Duru et al. (2013), examined pesticide residues in 33 of 145 fruit and vegetable samples which are sampled in İzmir in 2007-2008. In 74 of 145 analyzed samples was not found residue. In 30 samples were found residues below tolerance, in only 7 samples were found residues over-tolerance. The highest residues were found in the fresh grape, raisins and tomato samples.

Tatlı (2006) investigated the residue levels of pesticides commonly used in fresh fruit and vegetable samples (strawberry, tomatoes, artichoke, table figs, cherry, potatoes, peach, table grapes, olive) collected in the Aegean region and from human consumption areas and in the cultivation of dried food samples. In these samples, 50 pesticides with organic chlorine, organophosphorus insecticides and synthetic pyrethroids, strobulin and benzimidazole group were selected from fungicides. In tomato, artichoke, table figs, dried figs and potato samples were not found any detectable pesticide residues. And in the samples of other products at least one pesticide residue was found at detectable levels.

Residue quantities in the samples with residuals were evaluated according to Turkish Food Codex and EU MRLs and pesticide residues were found over 2.34% tolerance in agricultural products.

CONCLUSIONS

The usage of pesticides and the fate of this usage are always on the agenda in today's world, and it is also likely to remain like that. Because, as intensive pesticide use in traditional agriculture, there are controlled pesticide use in "Good Agricultural Practices" and natural pesticide use in organic agriculture. In many studies, toxicological risks and environmental risks of pesticide residues to human health have been researched as a result of excessive and unconscious pesticide use (Tiryaki, 2016). In this context, Good Agricultural Practices involves agricultural techniques which are environmentally sensitive, do not harm human and animal health, aim to protect natural resources, and ensure traceability and food safety.

With such production techniques, socially viable, economically profitable and sustainable agricultural production is targeted. Therefore, Turkey has the support of the Ministry of Food, Agriculture and Livestock to encourage the transition to good agricultural practices. When compared to conventional agriculture, these can be promising techniques for achieving healthy and reliable food.

REFERENCES

Anonymous 2017a. http://www.zmo.org.tr/resimler/ekler/52cf38361a20908_ek.pdf (accessed, March 25 2017).

Anonymous 2017b. https://ec.europa.eu/food/sites/food/files/safety/docs/rasff_annual_report_2015.pdf (accessed, March 25 2017).

Duru A.U., Örnek H., Burçak A.A., 2013. İzmir İli'nde satışa sunulan bazı meyve ve sebzelerde pestisit kalıntılarının araştırılması. I. Bitki Koruma Ürünleri ve Makinaları Kongresi, Bitki Koruma Ürünleri (Cilt I, Pestisitler), Bildiriler Kitabı: 59-69, 2-5.

Kandemir D., Kurtar E.S., Demirsoy M., 2015. Türkiye Sebze Fidesi Üretimindeki Son Gelişmeler, TÜRKTOB Türkiye Tohumcular Birliği Dergisi, No:4, 4.

Lehotay S.J., 2007. Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study, Journal of AOAC International, 2007 Mar-Apr, 90(2):485-520.

Tiryaki O., 2016. Türkiye'de yapılan pestisit kalıntı analiz ve çalışmaları, Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 32(1):72-82.

Toprak E., Gül A., 2013. Topraksız tarımda kullanılan ortam domates verimi ve kalitesini etkiliyor mu?, Tarım Bilimleri Araştırma Dergisi, 6 (2): 41-47.

TÜİK, 2016. Türkiye İstatistik Kurumu, www.tuik.gov.tr/ (accessed, March 25 2017).

Zengin E. and Karaca İ. 2017. Uşak İlinde Örtü Altı Üretimi Yapılan Domateslerdeki Pestisit Kalıntılarının Belirlenmesi, Süleyman Demirel University Journal of Natural and Applied Sciences, Published Online: 03.02.2017.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

BIOLOGICAL CONTROL OF TWO-SPOTTED SPIDER MITE IN PEPPER AND MELON CROPS CULTIVATED IN TUNNELS

Maria CĂLIN [1], Tina Oana CRISTEA [1], Silvica AMBĂRUȘ [1], Creola BREZEANU[1], Petre Marian BREZEANU[1], Marcel COSTACHE[2], Gabriela ȘOVAREL[2], Liliana BRATU[2]

[1]Vegetable Research and Development Station Bacau, Calea Bârladului Street, no. 220, Bacau County, Romania
[2]Research Development Institute for Vegetable and Flower Growing Vidra, Romania

Corresponding author email: sclbac@legumebac.ro

Abstract

The paper aims to present the attack characteristics and the results of biological control of two-spotted spider mite on pepper and melon crops cultivated in tunnels. The attack of two-spotted spider mite began in the first decade of June. The degree of attack of two-spotted spider mite on pepper plants was ascendant reaching 17.5% in the second decade of September. The degree of attack of two-spotted spider mite in melon was high, reaching 61.3% in the third decade of September. The predator, Phytoseiulus persimilis At.-H. (Arachnida: Mesostigmata: Phytoseiidae) was used to control the spider mite in peppers and melons. The variants utilized in our experiment were as it follows: V1 - one release with 50,000 ex./ha; V2 - one release with 100 thousand ex./ha; V3 – one release with 150 thousand ex./ha; V4 - Untreated control. The releases of Phytoseiulus persimilis has reduced the two-spotted spider mite degree of attack (DA%) on pepper in late August to 2.3% in V1, 2.1% in V2 and 1.7% in V3. In September the DA% was below 1% in all the three variants. In the untreated control, the pest degree of attack level increased from 9.5% in the first decade of August, up from 17.5% in the 2nd decade of September, and then began to decline. In melons, the releases of Ph. persimilis have reduced the attack degree in the third decade of August at 1.8% in V1, 1.9% in V2, 1.5% in V3. In all three variants, the degree of attack in September was less than 1%. In V4 (untreated control), DA% increased from 4.3% in the first decade of August, to 61.3% at the end of September.

***Key words**: biological control, Phytoseiulus persimilis, two-spotted spider mite, pepper, melon.*

INTRODUCTION

The two-spotted spider mite - *Tetranychus urticae* Koch is one of the main pests of vegetable crops grown in greenhouses and tunnels (Dalpe, 2002; Zhang, 2003; Calin, 2005; Herrmann et al., 2011).
The pest is polyphagous, that is attacking 1110 host species (Herrmann, 2017), in the field, greenhouse and tunnels. In our country, this pest attacks crops of eggplants, peppers, cucumbers, beans, tomatoes, etc. Pest attack is higher in crops of eggplants and cucumbers and less in other species (Candea, 1984). The spider mites have caused little visible damage to the leaves and induced direct defense responses. According to Merjin et all., 2004 after two-spotted spider mite attack the proteinase inhibitor activity had doubled and the transcription of genes involved in jasmonate-, salicylate-, and ethylene-regulated defenses had been activated. On day four, proteinase inhibitor activity and particularly transcript levels of salicylate-regulated genes were still maintained. In addition, genes involved in phospholipid metabolism were up-regulated on day one and those in the secondary metabolism on day four. Although transcriptional up-regulation of the enzymes involved in the biosynthesis of monoterpenes and diterpenes already occurred on day one, a significant increase in the emission of volatile terpenoids was delayed until day four.
The emergence of resistance to chemical acaricides, the negative effect of chemicals on useful fauna and pesticide residues determined the usage of predator mites, as *Phytoseiulus persimilis* Athias-Henriot for the control of two-spotted spider mite (Lenteren, 2003 and 2012).
This predator arrived accidentally in Germany from Chile in 1958. Anticipating their effectiveness in pest control in 1960 research began in the UK, Netherlands, Canada, USA etc. in order to determine the influence of this predator in the control of two-spotted spider mite

at various vegetable and flower crops (Rojas, 2010). The obtained results were exceptional and passed to currently reared *Ph. persimilis* (Shih 2001; Bolckmans, 2007) and its use in greenhouses and tunnels for control of two-spotted spider mite.

MATERIALS AND METHODS

During 2015 - 2016 years, experiments in tunnels were performed at Vegetable Research-Development Station Bacau - Romania, in order to study the two-spotted spider mite attack in pepper and melon crops and evaluate the effectiveness of biological control using *Phytoseiulus persimilis* predator. The relevant results from 2016 are presented in this paper.

1. Study of two-spotted spider mite attack in pepper and melon crops in tunnels

The observations were made every 10 days from May to September in Control.

For the attack estimation we used the following indicators:

- Frequency of attack (F%),
- Intensity of attack (I%),
- Degree of attack (DA%).

2. Biological control of pest.

The predatory mite *Phytoseiulus persimilis* At.-H. (*Arachnida, Mesostigmata, Phytoseiidae)* was used for biological control of pest.

The effectiveness of this predator in control of two-spotted spider mite - *Tetranychus urticae* Koch. was studied in pepper collection of cultivars and melon in tunnels. When the degree of attack of mites exceeded in pepper 9.5% the predatory mites were released in the 3 variants. The trial was done in the summer period and early autumn. The average highest day temperature was between 28-32°C with peaks up to 40°C.

The trials of *Ph. persimilis* for the two-spotted spider mite control were at the following release rates:

- V1 – 50,000 mites/ha;
- V2 – 100,000 mites/ha;
- V3 – 150,000 mites/ha;
- V4 - Control.

When the degree of attack of two-spotted spider mites exceeded in melon 4.3% the predatory mites were released in the same 3 variants as mentioned above.

A variant area was 45 square meters.

The effectiveness of the predator releases was determined by decadal observations of the attack on the plant and monitoring the pest population of mite in August and September.

The effectiveness evaluation of *Ph. persimilis* on the two-spotted spider mite was performed by the Sun - Shepard method.

RESULTS AND DISCUSSIONS

1. Study of two-spotted spider mite attack in pepper and melon in tunnels

The dynamic of the degree of attack of two-spotted spider mite on pepper is showed in figure 1.

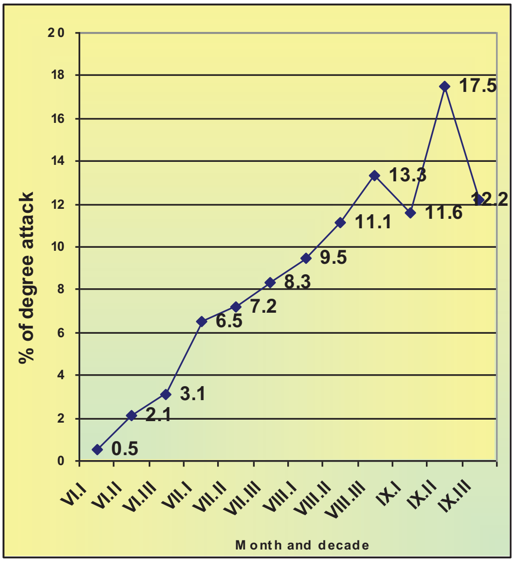


Figure 1. The attack of two-spotted spider mite in pepper

As shown in previous figure, the degree of attack in pepper has an ascendant dynamics. It started in first decade of June and increased to 17.5% in second decade of September. Then attack of pest begins to decrease reaching to 12.2%.

The dynamic of the degree of attack of the two-spotted spider mite on melon is presented in figure 2. The attack started in the first decade of June. The attack level was low until the first decade of August. Then the degree of attack had an ascendant dynamic from 4.3% in the first ten days of August, to 61.3% in the last decade of September. Under these circumstances it was necessary to apply measures for pest control, harvesting melons being carried out by the end of September.

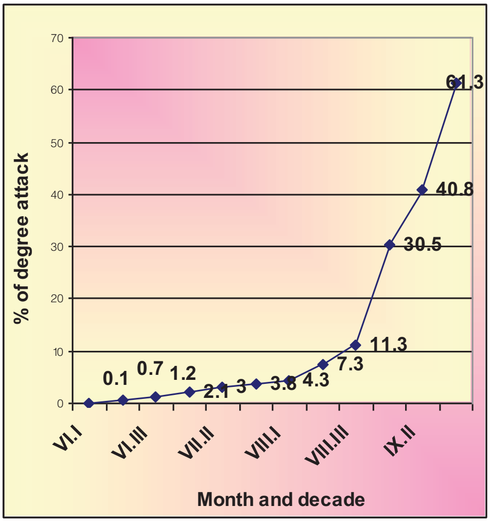


Figure 2. The attack of two-spotted spider mite in melon

2. Biologically control of pest.

The degree of attack (DA%) of two-spotted spider mite decreased in all 3 variants of *Phytoseiulus persimilis* applications - table 1.

Table 1. The degree of attack of two-spotted spider mite on pepper and melon plants

No. variant	DA% in month and decade					
	August			September		
	I	II	III	I	II	III
Pepper						
V1	13.5	9.5	2.3	0.7	0.4	0.1
V2	11.9	9.6	2.1	0.5	0.1	0.1
V3	9.0	6.6	1.7	0.2	0.1	0.1
V4	9.5	11.1	13.3	11.6	17.5	12.2
Melon						
V1	3.4	2.5	1.8	1.7	0.7	0.7
V2	6.1	4.5	1.9	0.8	0.7	0.7
V3	5.9	3.8	1.5	0.8	0.7	0.7
V4	4.3	7.3	11.3	30.5	40.8	61.3

The degree of two-spotted spider mite attack in V1 was reduced from 13.5% (first decade of August) to 9.5% (2nd decade of the month), 2.3% (3rd decade of August and less than 1% in September).

In V2, DA% had the same decreasing trend, which is below 1% in September. The V3, where the release rate was 150 thousand ex./ha had the lowest values of DA% (6.6% and 1.7% in August and in less than 1% in September, table 1). In control variant (without releases), the degree of two-spotted spider mite attack increased from 9.5% in the first decade of August, up from 17.5% in the 2nd decade of September, then began to decline. The effectiveness of the release was up to 98% in variants with release rate of *Ph. persimilis* at the end of September (Figure 3).

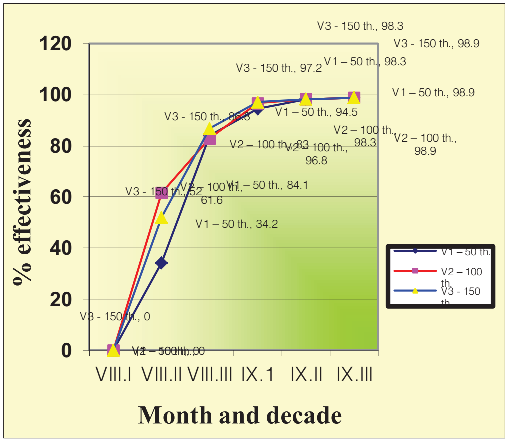


Figure 3. Effectiveness of *Phytoseiulus persimilis* releases in control of two-spotted spider mite on pepper crop

At melon, the pest degree attack was low (3.4% in V1, 6.1% in V2, 5.9% in V3 and 4.3% in control) in first decade of August.

After *Ph. persimilis* releases, the DA% decreased in the second decade of August as follows: V1 - 2.5%, V2 - 4.5%, V3 - 3.8%.

Descendant DA% continued for all three variants, reaching values below 1% in September. In V4 (control without predators release), the degree of attack had a sharp upward trend rising from 4.3% in the first ten days of August, to 61.3% in the last decade of September.

The effectiveness for all three releases rates of *Ph. persimilis* was up to 98% in all variants at the end of September (Figure 4).

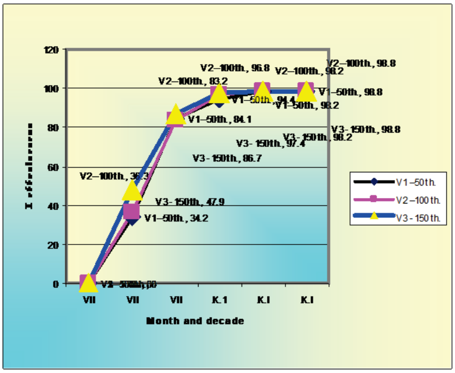


Figure 4. Effectiveness of *Phytoseiulus persimilis* releases in control of two-spotted spider mite on melon crop

CONCLUSIONS

Study of two-spotted spider mite attack on pepper and melon crops in tunnels

The degree of attack in pepper has an ascendant dynamics. It started in first decade of June and increased to 17.5% in second decade of September. Then attack of pest begins to decrease reaching to 12.2%.

In melon the attack started in the first decade of June. The attack level was low until the first decade of August. Then the degree of attack had an ascendant dynamic from 4.3% in the first ten days of August, to 61.3% in the last decade of September.

Biologically control of pest. The degree of two-spotted spider mite attack in V1 was reduced from 13.5% (first decade of August) to 9.5% (2nd decade of the month), 2.3% (3rd decade of August) and less than 1% in September. In V2 DA% had the same decreasing trend, which is below 1% in September. The V3, where the release rate was 150 thousand ex./ha had the lowest values of DA% (6.6% and 1.7% in August and in less than 1% in September). In control variant (without releases), the degree of two-spotted spider mite attack increased from 9.5% in the first decade of August, up from 17.5% in the 2nd decade of September. The effectiveness of the release was up to 98% in variants with release rate of *Ph. persimilis* at the end of September.

At melon, the pest degree attack was low (3.4% in V1, 6.1% in V2, 5.9% in V3 and 4.3% in control) in first decade of August. After *Ph. persimilis* releases, the DA% decreased in the second decade of August as follows: V1 - 2.5%, V2 - 4.5%, V3 - 3.8%. Descendant DA% continued for all three variants, reaching values below 1% in September. In V4 (control without predators release), the degree of attack had a sharp upward trend rising from 4.3% in the first ten days of August, to 61.3% in September.

The effectiveness for all three releases rates of *Ph. persimilis* was up to 98% in all variants at the end of September.

REFERENCES

Bolckmans K.J.F., 2007. Mass-rearing phytoseiid predatory mites. Proceedings of the Working Group AMRQC, C. van Lenteren, P. DeClercq, and M. W. Johnson, Eds., Bulletin IOBC Global, vol. 3, 12–15.

Calin M., 2005. Ghidul recunoaşterii şi controlului dăunătorilor plantelor legumicole cultivate în agricultură biologică, Ed. TIPOACTIV, ISBN 973-87136-3-3.

Candea E., 1984. Daunatorii legumelor si combaterea lor. Ed. Ceres, Bucuresti.

Herrmann I., Berenstein M., Amit Sade, Arnon Karnieli, David J Bonfil, Phyllis G Weintraub, 2011, Evaluation of spider mite damage to greenhouse pepper leaves by spectral assessment. Ptocedings '7th EARSeL Workshop on Imaging Spectroscopy' April 11-13, 2011, Edinburgh, Scotland, UK.

Lenteren J. C. van, 2003. Commercial availability of biological control agents," in Quality Control and Production of Biological Control Agents: Theory and Testing Procedures, J. C. van Lenteren, Ed CABI, Oxon, UK.,

Lenteren J. C. van, 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake," BioControl, vol. 57, no. 1, 1–20.

Rojas M. G., J. A. Morales-Ramos, 2010. Tri-trophic level impact of host plant linamarin and lotaustralin on *Tetranychus urticae* and its predator *Phytoseiulus persimilis*. Journal of Chemical Ecology, vol. 36, no. 12, 1354–1362.

Shih C.I.T., 2001. Automatic mass-rearing of *Amblyseius womersleyi* (Acari: Phytoseiidae), Experimental and Applied Acarology, vol. 25, no. 5, 425–440.

Dalpe S. 2002. Pests of Greenhouse Sweet Peppers and their Biological Control. Alberta.ca Agriculture and Forestry,

Zhang Z. Q, 2003. Mites of Greenhouses Identification, Biology and Control, CABI.

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

WHY POPLAR AND NOT KAKI. PUZZLES, PLANTS, MEMORY AND STORYTELLING GARDENS

Alexandru MEXI

University of Bucharest, Faculty of Letters, Center of Excellence
in Image Study, Space, Image, Text, Teritorry Doctoral School,
5 Mihail Moxa Street, 010961, Bucharest, Romania

Corresponding author email: alx.mexi@gmail.com

Abstract

Throughout the history of mankind, vegetation played a crucial role in mythology, philosophy, natural science and gardening. In what concerns garden design, choosing a specific species of plants served different types of purposes and helped to create magnificent storytelling landscapes. Associated with virtues, gods and goddesses, feelings and emotions, etc. plants defined almost every story behind garden design since the ancient times to the present day. However, sometimes, a garden was built around existing plants and this is why such particular examples of species of trees, shrubs or flowers became so much more important in the history and philosophy of a garden than those that were planted afterwards, according to a specific and predetermined pattern. One such example is represented by Queen Marie of Romania's gardens at Balchik and their famous old bended white poplar (Populus alba), an ancient tree that had became one of the reasons for which Queen Marie decided to build her most renowned gardens at Balchik. Today however, the old poplar was replaced by a kaki tree (Diospyros kaki) and by this what may seem to be a minor modification, an entire part of the garden's meaning was profoundly altered. To this end, the following paper aims to emphasize the importance of plant species in what concerns the story and allegories of gardens, focusing on the examples offered by the Balchik gardens.

Key words: *plant species, garden history, Queen Marie of Romania, Balchik, memory, poplar, lily.*

INTRODUCTION

Queen Marie of Romania, an iconic personality of modern Romania was deeply in love with art, and one of the arts she treasured most was the art of gardens. Her love for plants and garden design is no secret to any historian or landscape architect interested in her life and achievements, as she personally expressed her feelings in numerous occasions throughout her entire life, but also in her written memories that were published (partially) after her death. However, her most renowned gardens were the ones she built by the sea side at Balchik, and one of the reasons she chose this place to build her garden-Paradise was an old bended white poplar she saw and fell in love with during one of her travels in 1924 (see Regina Maria a României, 2014). By this means, the white bended poplar became a symbolical pillar around which the gardens were built (Figure 1) and his presence in the overall image of the gardens became a crucial element that solely described an important part of the memory of the gardens. However, this memorial element and others alike were replaced by different other types of vegetation (more or less alike) and although this may seem to be a minor modification, it is actually altering the general concept of the gardens that Queen Marie created by the seashore.

Figure 1. The old bended poplar and the Queen's castle, approx. 1930
(Source: ANIC, Fond Fototeca, Format 1, 306-1)

To this end, this research is focusing on the importance of plant species in what concerns allegorical and storytelling landscapes, emphasizing their role in the history and memory of a historical ensemble, offering detailed historical references about why some particular species of plants were specifically chosen by the garden's owners and/or the landscape architects to be used in the landscapes they designed.

MATERIALS AND METHODS

In order to understand the meaning and the importance of plant species in the design of the gardens at Balchik one must first understand the history and the philosophy behind the design used by Queen Marie and her landscape architects for Her Majesty's gardens by the Black Sea. To this end, this chapter will focus on analyzing the history of the Balchik gardens by studying both written sources and archives and historical images, focusing on how and why some particular plants were preferred and used in some parts of the gardens in order to create symbolical and allegorical stories.

Short history

Queen Marie of Romania dedicated a major part of her private time to her gardens and, in April 1930 she even published an article in a Romanian magazine, namely "*Boabe de grîu",* in which she described her most beloved "dream houses" (Maria, 1930). A large part of this article was dedicated to her house and gardens by the seashore at Balchik (nowadays on Bulgarian territory), and one of the details she mentioned repeatedly was an "*old tree that was hanging by the sea side like a gem from the pick of a ruined wall*" (Maria, 1930, pp. 70-71). This tree was a bended ancient white poplar (*Populus alba*) to which the Queen fell in love with.

The sovereign visited Jean Chrissoveloni's property in Balchik on the 9th of October 1924, and when she saw "*an immense poplar, a giant perched right above the sea, blooming form a small terrace on the beach*" (Regina Maria a României, 2014, p. 416) she decided to buy this property and build herself a small mansion surrounded by terraced gardens similar to the ones she saw in Italy. Since she first saw Jean Chrissoveloni's property and before she even contacted him to ask him to sell her this land, the queen envisioned how both her house and part of her gardens would look like, picturing a house similar to an old house from Buhari and a series of "spanish", "turkish", "italian" and "rustic" gardens covered with multiple species of flowers (Regina Maria a României, 2013).

In time, she will buy both Chrissoveloni's property and many more adjacent ones, creating a maze of gardens stretching along the coastline. Some of the gardens will be designed for and named after Queen Marie's children and her husband, thus transforming a large part of the complex into a memorial landscape in which her house, the *Tenha Yuhav*, will be enclosed in an allegorical line of special, family gardens. Besides these gardens, the sovereign will design a garden for her son in law, King Alexander Ist of Yugoslavia, her nephew Tomislav of Yugoslavia, for Gaetan Denize (Queen Marie's personal secretary) etc.

The queen will also build two very interesting gardens that she will name the Garden of Gethsemane and Allah's garden. These gardens, but also the other ones mentioned before, were probably inspired not only by different classical sources, but also by the Baha'i faith (see Constantin, 2007), a religion Queen Marie became fond for in the 20th century (Marcus, 2000). However, the relationship between the gardens at Balchik and their correspondences with the Baha'i faith believers is not part of this study, but it is aimed to become a future topic of research.

The gardens at Balchik will represent a crown jewel for the queen's creations because unlike other architectural and landscape ensembles that belonged to her, Balchik would have no imaginative boundaries and they "*will support the idea of evasion, of losing oneself in nature*" (Constantin, 2007, p. 166) and most of all because their design will bring together the Queen's childhood love for the sea, the love for plants and gardening, the love for her children, her knowledge and passion for arts and religious or profane symbols (such as Christian altars, statues of saints or mythological heroes etc.) etc. To this end, the landscape ensemble by the seashore will become something similar to an *imago mundi* for the Queen, a both physical and symbolical world that revolved around the *Tenha Yuhav* (the Quiet Nest) and gathered all of Queen Marie's love, loves, passions and childhood desires. (after Mandache, 2014, p. 64).

In all this complex allegorical landscape, two plants stand out and they define one of the reasons for which the queen chose Balchik to build her personal "Paradise" and the way she chose to be remembered after her death. The two plants are represented by the old bended white poplar and by the lily path that she planted in order to leed to her sanctuary, namely to the Stella Maris chapel.

Plants and historical references

Although every particular garden from the Balchik ensemble was planted with particular species of plants in order to underline a specific allegorical message that most of the time alluded to one of the queen's passions or family members (as we stated before), two plants defined 'the birth and the end of the garden': the ancient poplar and the white lilies.

The white poplar.

The queen was so fond of this tree she found during her 1924 visit that she repeatedly mentioned it in many of her notes, articles and daily records and moreover. She thus decided to place a white marble throne right under its shadow, transforming the tree into something similar to a royal emblem (Figure 2).

The tree was integrated in the garden that bore the name of the Queen's oldest daughter, Elisabeth. The poplar was also in close proximity to Carol's terrace (her firstborn child and the future King Carol II) and to the main residence, the *Tenha Yuhav,* representing the only large tree in their proximity.

Figure 2. The old bended poplar, the throne and Elisabeth's terraced garden in the background, approx. 1927-1930
(Source: ANIC, Fond Fototeca, Format 1, 565-3)

Although both European classical mythology (see Chevalier and Gheerbrant, 1995) and the Romanian beliefs (see Simion-Florea, 2008) consider poplars to be part of a small category of plants regarded as negative symbols, it is most probably that the ancient white poplar from the terraced gardens at Balchik bore no specific symbolism, but rather it was regarded as one of the elements most representative for the landscaping complex. The poplar, in the case of the Balchik ensemble, thus became a symbol for the birth of the gardens.

The lily road.

If we can accept the fact that the secular poplar bore no symbolical meaning, we can not state the same thing for the lilies planted by Queen Marie on the alley that connected the Temple of the Waters or *Suleiman Lei*, to the Stella Maris chapel. They were specifically chosen by the Queen to be planted on the way to the place she wished her heart to be laid after her passing. As we shall see, if the poplar was an element that the sovereign desired to keep and integrate in her gardens, transforming it into a kind of symbol for the birth of her architectural and landscaping personal Paradise, the lilies were chosen for a number of reasons that dealt with symbolism, mythology and personal beliefs.

Queen Marie's love for flowers is widely known and even she stated in her daily notes that she had an even more increasing love for them as she grew older: "*Flowers play a crucial role in my life, more overwhelming as the years pass* [...]" (Regina Maria a României, 2014, p. 431), but one of the plants she was fond to more than for others was the white lily, also known as the Royal lily or Madonna's lily (*Lilium regale*).

The reason why the lily meant so much for the Queen as she decided to place it on the road that lead to her heart was that, when she first saw it in childhood while she was at the Swiss Cottage on the Isle of Wight, she was fascinated by its beauty and by its perfume: "*It is a whole world in Madonna lily's scent, something biblical, something legendary, almost beautifully ireal. More than that, it is so tall and graceful, so shiny, as it seems that its petals spring light*" (Mandache, 2014, p.14, quoting from Marie, Queen of Romania, 1936, vol. I). So, as we see, the lily was part of a memory so pleasant and so deeply rooted in Queen Marie's memory that it is obvious why this particular plant would be chosen to lead the way from the Temple of the Waters to the Gethsemane garden and to the Queen's heart. However, not just memory played a crucial role in choosing the lily as the most representative flowering plant for this symbolical "assignment".

It is considered that the queen was no stranger to the significance of the symbols she chose for different purposes and reasons in all her artistic works. To this end, the lily might have been used both because it was regarded as an emblematic symbol of Virgin Mary, the Queen's patron (see Constantin, 2007) and because the lily is one of the most complex symbolical plants, representing innocence, purity, passionate love, glory, Christian belief, mystical grace etc. (see Chevalier and Gheerbrant, 1995) - all traits that a Queen should posess.

All these characteristics and symbolical meanings of the lily along with the queen's childhood love for this particular flower represented more than enough reasons for choosing the *Lilium regale* as the flower to be planted on the road that lead to the queen's heart. They mark "the end of the road" for the Queen but also a new possible and hopefully bright future inspired by Marie of Edinburgh. (Figure 3)

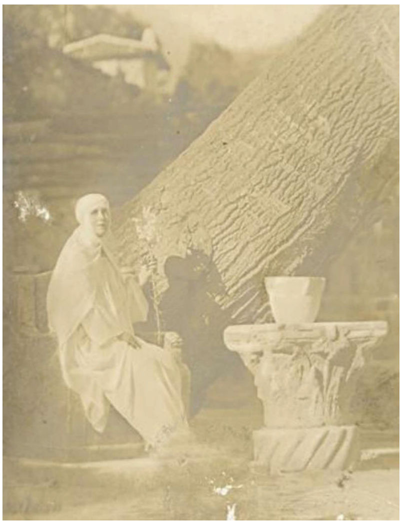


Figure 3. The Queen holding a lily and sitting under the ancient poplar's shadow (approx. 1935)
(Source: ANIC, Fond Fototeca, Format 1, 218-2)

On the 24th of July 1938, Queen Marie died and was buried alongside her husband at Curtea de Argeş. However, according to her will, her heart was removed from her chest and was placed in a silver box within a golden box with precious gems and was sent to be laid at Balchik in the small Stella Maris chapel.

The lily road was welcoming her and all those who wanted to come to her heart and pay her an homage, the exact way she had planned: "*During an abundant lifetime, so many came to my heart, and so I wish that the even dead still feel them coming to it, along the path of lilies, that was my joy and pride" (Mandache, 2014, quoting from archival sources - ANR)* - (Figures 4 and 5).

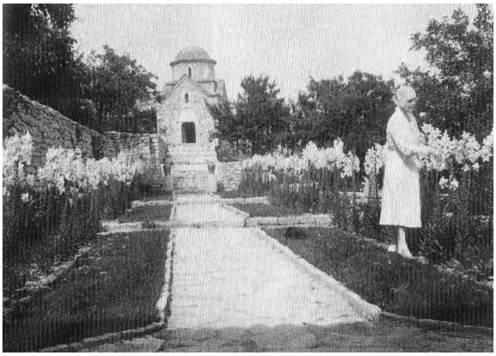


Figure 4. The Queen, Stella Maris chapel and the lily path (approx. 1930-35)(Source: MNP archives)

Figure 5. The Queen and Stella Maris chapel, approx. 1935 (note: the lily parh is behind the photographer)
(Source: ANIC, Fond Fototeca, Format 1, 226-5)

From the private gardens to the public botanical park

After the Queen's death, the entire ensemble became a topic of disputes between the Romanian and the Bulgarian governments and since the 1940s and up until the 21st century few details are known about how the gardens have changed over time, how, what and why did some particular terraces were redesigned etc. However, we will skip this historical period of political and economical debates between the two nations, and we will focus on the present image of the gardens.

Although we will not discuss political or economical matters, it is important to mention that after Queen Marie's death, the gardens started being slowly opened to the public, but most of the time, as historical documents and contemporary researches show, the visitors were often very aggressive and they didn't treated the gardens properly and with respect: "*These visits are becoming even more numerous and the people are wandering the entire park without being supervised by anyone, throwing food everywhere, picking flowers, climbing on everything and leaving after getting bored.*" (after Mandache, 2014 quoting from archival sources - AMAE).

In the late 1900s, the Balchik complex became a guest house ensemble and this might also be one of the reasons why the gardens became even more degraded.

Nowadays, Queen Marie's gardens at Balchik are considered to be botanical gardens due to their impressive plant collections. However, they were never designed as such and they where never intended to become botanical gardens. They do not even follow the prescriptions and the botanical rules that a botanical garden needs to follow, but because of their indigenous and exotic plant collections they are perceived and presented as such.

Regarding the historical vegetation, a series of *in situ* and archival researches have to be done in order to reveal which parts of today gardens still holds the historical plant species and which do not. However, regarding the old white poplar and the lily path we can observe that they were replaced by a kaki tree (Figures 6 and 7) and by groups of irises,and the reasons why such modifications were made are still uncertain and unclear.

Figure 6.The ancient bended poplar (approx. 1935-40)
(Source: MNP archives)

Figure 7. The young kaki tree, 2015
(Source: author)

RESULTS AND DISCUSSIONS

Since 1924 and up until 1938, the gardens at Balchik were in a constant change and upgrading of both their plant collections and of their symbolism. Although many parts of the ensemble went through several changes, some parts remained unchanged and the Queen herself explained the reasons why she chose these particular parts of the gardens not to be modified. Thus, the old bended white poplar, due to the fact that it was one of the reasons why the queen chose these grounds to build her "paradise" gardens, it became an iconic vegetal component of the royal landscape ensemble by the seashore. In what concerns the lily path, this was one of the few plant compositions for which the queen explained the reasons why she decided to design it as such.

As we see, a series of elements or parts of the gardens were very meticulously thought through by the Queen and by her gardeners and landscape architects and these are the reasons why they became iconic for the history and symbolism of the Balchik landscaping complex. Each plant, each sculpture, each vase and each building had a purpose and a role in the garden's iconography. They were part of what Diana Mandache is naming a "Fool's Paradise" or the "Illusory Paradise" because the gardens built by the Queen were nothing more for the sovereign than an ideal world created on the basis of a "happiness based on an imaginary blissful state, designed far from the unforgiving realities; a place of refuge and silence" (Mandache, 2014, p. 40). Due to the fact that every detail of the gardens was meticulously conceived in order to serve such purposes, it was important to keep, conserve or restore it/them in order to maintain a particular symbolism and allegorical story that defined the iconographical statement of the gardens.

Regarding the old bended white poplar and the lily path, as we have seen, their role in the history and iconography of the gardens was very important and, as he have already stated, they were physical and symbolical statements of the garden's birth and "afterlife". The two species were part of a complex landscaping microcosm defined as an "Illusory Paradise" and if we may compare the Balchik ensemble with a puzzle we can see that if we remove or change even a single piece, we cannot obtain the desired image. Thus, the new species of plants that took the place of the historical ones create a different image and distort Queen Marie's vision.

Indeed, plants have a certain lifespam and we cannot have hoped that the ancient poplar would have lasted for eternity, but when it was time to cut it, a new similar species of tree should have taken its place and continue the story that the old poplar was telling, and not change it with a different species of tree that may or may not create a different storyline. The same arguments go for the lily path as well, only that when it comes to the lilies one may decide that since the queen's heart is no longer at Balchik, then it is probably no need to keep the symbolical lilies. However, this is an argument that must be very well sustained.

CONCLUSIONS

In conclusion, even if we refer to the examples given by the Balchik gardens or to any other similar ones, we can conclude that eliminating or replacing certain elements from a garden, whether they are natural, mineral or manmade, is also modifying certain paradigms and concepts and it alters the iconographical puzzle and the allegorical messages that a certain garden was designed to create. If we refer only to the Balchik ensemble, we can see that, by replacing the ancient poplar with a young kaki tree and by replacing the lilies with irises, the beginning and the end of the story that Queen Marie decided to create by the seaside are altered.

However, there are many more other similar examples of gardens where the original species of plants were replaced by others, but this is not necessarily a bad decision. Replacing certain species of plants in historical gardens had, throughout history, both benefic and destructive consequences. To this end, many more similar examples could be given, and only by remaining in the relatively same geo-political area, we could give the Cişmigiu garden from Bucharest as an example where these types of actions had both good and bad consequences (Mexi and El-Shamali, 2015).

To this end, we can state that, if possible and unless there are technical or environmental issues that are demanding this, the original pieces, whether they are natural or manmade, should be kept, conserved and restored in order to keep a continuous and coherent allegorical and symbolical storyline which a garden was meant and designed to reflect.

ACKNOWLEDGEMENTS

I would like to thank Assistant professor *Elisabeta Dobrescu* from the University of Agronomic Sciences andVeterinary Medicine in Bucharest for the guidance and feedback provided for the second part of this research during the „Crinii Reginei Maria" workshop held in Balchik in 2015.

REFERENCES

***, 2013. Casele de vis ale Reginei Maria - Castelul Balcic. Album: Muzeul Naţional Peleş.

Arhivele Muzeului Naţional Peleş, (MNP). Fond Fototeca.

Arhivele Naţionale Istorice Centrale, (ANIC). Fond Fototeca.

Arhivele Naţionale Istorice Centrale, (ANIC). Fond Castele şi palate.

Constantin, M., 2007. *Palate şi colibe regale din România.* Compania, Bucharest.

Chevalier J., Gheerbrant A., 1995. *Dicţionar de simboluri* (Vol. I). Artemis, Bucharest.

Chevalier J., Gheerbrant A., 1995. *Dicţionar de simboluri* (Vol. II). Artemis, Bucharest.

Chevalier J., Gheerbrant A., 1995. *Dicţionar de simboluri* (Vol. III). Artemis, Bucharest.

Mandache, D., 2014. *Balcicul Reginei Maria.*Curtea Veche, Bucharest.

Marcus, D. L., 2000. *Her Eternal Crown - Queen Marie of Romania and the Baha'i faith.* George Ronald, Oxford.

Maria (Regina României), April 1930. Casele mele de vis. *Boabe de grâu*, pp. 65-78.

Mexi A., El-Shamali S. A., 2015. Cismigiu garden in between original design and further transformations – a comparative study on a continuously redesign process. In Scientific Papers. Series B, LIX, pp. 355-364.

Regina Maria a României, 2013. *Însemnări zilnice* (Vol. VII). (S. I. Racoviceanu, Trans.) Polirom, Bucharest

Regina Maria a României, 2014. *Însemnări zilnice* (Vol. VI). (S. I. Racoviceanu, Trans.) Polirom, Bucharest

Simion Florea, M., 2008. *Botanica poporană română* (Vol. I).Muşatinii, Suceava.

Simion Florea, M., 2010. *Botanica poporană română* (Vol. II). Editura Academiei Române, Suceava.

Simion Florea, M., 2010. *Botanica poporană română* (Vol. III). Editura Academiei Române, Suceava.

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CHARACTERISTICS OF GROWTH AND DEVELOPMENT OF THE SPECIES *SPARTIUM JUNCEUM* L. IN THE REPUBLIC OF MOLDOVA

Ion ROȘCA, Elisabeta ONICA, Alexei PALANCEAN

Botanical Garden (Institute) of the Academy of Sciences of Moldova,
18 Padurii Street, Chisinau, Republic of Moldova

Corresponding author email:onicaelisaveta@yahoo.com

Abstract

This article describes the characteristics of growth and development of the species Spartium junceum L. (weaver's broom, Spanish broom), which can be used in various branches of the national economy. The most effective method of propagation was sowing the seeds, which had been previously treated hydrothermally with hot water of 70°C for 16 hours and with 0.01% gibberellin solution for 24 hours, in trenches, in spring – in April or May.

Key words: *generative propagation*, *Spartium junceum L.*

INTRODUCTION

In relation to climate change, dangerous processes of vegetation degradation and worsening conditions for the development of woody plant species occur, making the continuous mobilization, conservation and rational use of biodiversity more necessary than ever.

The family *Fabaceae* L. includes trees, shrubs, subshrubs, perennial and annual grasses. Over 700 genera and 17,000 species, spread almost all over the globe, are part of this family.

The genus *Spartium* L. includes only one species. *Spartium junceum* L. (common names: weaver's broom, Spanish broom) was introduced in the Republic of Moldova in 1867 [1, 2]. Bast fibres extracted from this plant may be used as raw material for making thick fabrics and weaving baskets. This ornamental, melliferous shrub has been ranked "A" for its decorative qualities and can be cultivated in central and southern districts of Moldova, but, for unknown reasons, it isn’t widely used. In the north of the country, this species is affected by low temperatures.

It is native to the Canary Islands and the Mediterranean Basin. That is why we decided to study the peculiarities of growth, development and reproduction of this species, in order to use it in various sectors of national economy.

MATERIALS AND METHODS

Plants of 5-6 years-old that grew and developed in the collection of the Botanical Garden (I) of the ASM from Chisinau served as research materials.

Phenological observations were carried out according to methodical indications [5], in 2013-2016. The seeds, collected in August and September, were stored differently, cleaned by various methods and treated according to the methodology [3]. Prior to the incorporation in fine loose soil, the seeds were treated with hot water of 70°C for 30, 60 minutes and 16 hours, until the water chilled, and with 0.01% and 0.03% gibberellin solution - for 24 hours. The seeds were sown in two periods, in February-March, in boxes, in greenhouses, in a mixture of soil, sand and peat in a ratio of 2:1:1, and in April-May, depending on climatic conditions. The fruits and seeds collected during the years of research were analyzed by several morphological parameters, in the Dendrology Laboratory of the Botanical Garden (Institute) of the ASM.

RESULTS AND DISCUSSIONS

***Spartium junceum* L.** is an evergreen shrub, which grows up to 2-3 m tall. Its stems are erect, cylindrical, light-green, glabrous and leafless or with few leaves. The leaves are simple, narrow-lanceolate or lanceolate, glabrous or sparsely hairy, ephemeral, blue-green, 1-2.5 cm long. The growing season started, in 2014-2016, 10-15 days earlier than the multiannual average. The leaves developed in April-May (Table 1.). The stems remained green throughout the growing season until next spring. In the years of the research, the leaves fell in October. The flowers are papilionaceous, yellow, fragrant, 2-2.5 cm across, in erect terminal racemes. The calyx is bilabiate, the upper labium is double-toothed and the lower – undivided during flowering. The wings are longer than the keel (carina), the keel – acuminate or curved, the stamens – monadelphous, the anthers – hairy, the ovary – sessile, multiovulate, with linear style, slightly curved at the tip, elongated stigma. The fruits mature in August-September (Table 1).

Table 1. Phenological stages of the species *Spartium junceum* L.

Phenological stages	**2013**	**2014**	**2015**	**2016**
Bud swelling	18 April	1 April	6 April	2 April
Bud opening	20 April	9 April	25 April	10 April
Beginning of flowering	18 May	16 May	20 May	9 May
End of flowering	18 June	24 June	20 June	15 June
Beginning of fruit maturation	10 August	5 August	11 August	8 August
End of fruit maturation	10 September	3 September	6 September	5 September

The abundance of flowering and fruiting is in close correlation with the air temperature and the amount of rainfall during the respective stages. The flowers develop on annual shoots.

The fruits are polyspermous, linear pods, 4-8 (10) cm long and 0.5-0.7 cm wide, at first villous, then glabrescent. Depending on the climatic conditions in the years of research, the percentage of fruiting ranged between 48 and 65 %. Temperature fluctuations had a negative impact on the process of ontomorphogenesis of seeds and only half of them were viable, the rest - sterile. The viable seeds differed from the sterile ones in colour, size and weight.

Spartium junceum L. is an evergreen shrub, very decorative in the flowering stage, with fragrant flowers, which contain essential oil. It flowers and bears fruit from the age of 3-4 years. *Spartium junceum* L. grows fast in the first 3-4 growing seasons and reaches a height of 2 m, is light tolerant, drought resistant, grows on arid soils and develops well on slopes exposed to the sun. In cold winters, a part of the plant may be affected, but it recovers by producing root sprouts. After pruning, the number of shoots increases and the flowering stage starts later than usual. Hydrothermally treated seeds, sown in February-March in the greenhouse, germinated evenly in 15 days, while those sown in April-May germinated unevenly in 20-25 days. The germination of the seeds, treated with 0.01% gibberellin solution for 24 hours and sown in trenches, was more even and by 10-15% higher as compared to the untreated seeds. The 0.01% gibberellin solution influenced positively the germination capacity of seeds and the germination percentage, in the years of research, constituted 85-90%.

Before sowing, the seeds had been soaked in hot water for 16 hours, until the water cooled. Transplanting the 5-7 cm tall seedlings, obtained from the seeds sown in February-March, did not give the expected results. The seedlings obtained from seeds treated with hot water for 16 hours had a less developed root system as compared with those obtained from seeds treated with gibberellin. The seedlings obtained from seeds treated with 0.01% gibberellin solution for 24 hours, sown in trenches, in April-May, reached a height of 30-35 cm, by the end of the growing season, and had a well-developed root system.

These seedlings were planted in loose soil. The survival rate of seedlings was about 80-90%, depending on climatic conditions and compliance with the technology in the years of research. The weight of 1000 fruits was 350-420 g and the weight of 1000 seeds ranged between 14 and 30 g.

Analysing the data from Table 2, we can conclude that the fluctuations in temperature and the amount of rainfall in the years of research, 2013-2014, had a negative impact on the morphological parameters of fruits: the percentage of fruiting, the share of seeds in the mass of fruits, the number of viable seeds in a fruit. The lowest percentage of fruiting was recorded in 2015.

Table 2. Morphological characteristics of fruits of *Spartium junceum* L.

Morpho-biological characteristics	2013	2014	2015	2016
Weight of 1000 fruits, g	350	420	400	380
Weight of 1000 seeds, g	14	30	18	22
Share of seeds in the mass of fruits, %	4.0	7.14	4.5	5.8
Seed diameter, mm	4.0	3.0	4,0	4.0
Number of seeds in a fruit, units	11	15	12	16
Fruiting percentage, %	60	65	48	50
Peduncle length, mm	6.0	8.0	7.0	8.0
Fruit length, mm	75	73	65	81

The seeds were sown about 2 cm deep. The norm of pure and fertile seeds was 3-4 g per linear meter. Transplanting can be carried out in the first 2-3 growing seasons because the survival rate decreases by 40-50% as the plants get older. The well developed and deep root system hinders transplanting of mature plants in the field.

CONCLUSIONS

1. *Spartium junceum* L. is a heliophile, melliferous species, decorative in the flowering stage. It grows fast and is undemanding to soil, can be transplanted in the first 2-3 growing seasons.
2. The duration of the flowering stage of this shrub, the abundance of flowering and fruiting is in close correlation with the climatic conditions at the time.
3. The optimal method of propagation of *Spartium junceum* L. was sowing in spring, in April or May, the seeds that had been previously treated hydrothermally with hot water of 70°C for 16 hours and with 0.01% gibberellin solution for 24 hours.
4. *Spartium junceum* L. can be used as an ornamental plant in landscape architect-ture, planted in groups with other, taller species or along small alleys.

REFERENCES

Palancean A., 2015. Dendroflora cultivată din Republica Moldova. Autoref. tezei de doctor abilitat în ştiinţe biologice. Chişinău, 46 p.

Palancean A., Comanici I., 2009. Dendrologie (Asortimentul de arbori, arbuşti şi liane pentru împăduri şi spaţii verzi). Chişinău: F.E.-P: „Tipografia Centrală”, 519 p.

Palancean A.I., 2013.Reproducerea speciilor lemnoase (lucrare metodică). Chişinău.48 p.

Деревья и кустарники СССР,1958, М.-Л, Т. IV, с. 68-70.

Методика фенологических наблюдений в ботанических садах СССР,1972, V 113,с.3-8

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE INFLUENCE OF DIFFERENT ROOTING SUBSTRATES ON THE ROOT SYSTEM OF *ABUTILON HYBRIDUM* HORT. NEW PLANTS OBTAINED BY CUTTINGS

Diana VÂŞCĂ-ZAMFIR, Mihai Cristian POMOHACI, Leonard ILIE

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: dianazamfir2000@yahoo.com

Abstract

The purpose of the research was to identify to what extent rooting substrates used for vegetative propagation on commercial plant Abutilon hybridum, influence the defining parameters for the future decorative potential of the plants. It has been tested a number of substrates defined as classical for two different rooting cuttings shoot (the segment and peak), aiming the evolution of the roots. For statistical interpretation of data it was developed an indicator that reflects the two types of measurements: percentage of rooting (%) and the average length of root cuttings (avg) for each type of substrate, called ITPS (I = Rooting T = Segment, P = Peak, S= Shoot).

***Key words**: Abutilon, Alpha Cronbach, cuttings, substrate.*

INTRODUCTION

Abutilon hybridum is a species originating in tropical and subtropical areas of southern America. This species is a vigorous shrub that can reach up to 1.5 meters high. Flowering occurs in summer, with fine flowers, red colored appear to be suspended like bells (Fanghua Niu et al., 2013).

Abutilon plants are eminently decorative indoor plant, grown in container system, but it can be seen also in the outdoor spaces in summer. Here are some of the reasons why the species is becoming more popular: it well recover the sunny places present in front of windows, supports very well logging, maintenance work are relatively simple for this plant. Because shoots are long, lean and suspended, plants can be used in hanging baskets or any other medium that allows it to manifest its pendent character.

The propagation by cuttings, regardless of their type, the organogenesis reproduces normal polarity plant due to natural auxines movement to basal pole, where the roots are formed. Regeneration of new individuals of plant fragments is governed by the complex interplay of hormones and other endogenous factors. The rooting process is stimulated by the presence of buds and leaves (H.T. Hartmann et al., 1983, quoted by Burzo et al., 2004). Also from cuttings are true regenerate's decorative features of the mother plant (Şelaru, 2005).

Whether rooting substrates are formed from natural materials (peat, sand) or artificial materials (perlite, vermiculite etc.) used alone or in mixtures, they must be sterile, breathable, have good ventilation and a good retention of water capacity and shape stability (Davidescu et al., 2001; Şelaru, 1995, 2005).

Worldwide a series of works are dealing with items on rooting plant *Abutilon hybridum*, in terms of the influence of certain factors on cuttings rooting. Either it been determined the irrigation norm for certain types of substrates (60% peat, 40% perlite) during cuttings rooting on *Abutilon* and *Lantana* (Kim and van Iersel, 2009) either is determinate the influence on daily lighting scheme on cuttings rooting placed on a substrate consisting of 80% peat and 20% perlite (Currey et al., 2012).

Usually the literature contains references to the local substrates used because of the specific location of the conducted research. Thus in a research that studies the substrate effect on the percentage of survival and growth of plants Jojoba (*Simmondsia chinensis*) obtained from cuttings at Jojoba Naturals Company greenhouse and shade house, Sana'a Yemen it been used in the first experiment a addition of

peat and sand substrates that can be easily obtained at the place where research is conducted (Ahmed M. EeD, 2016).
For this reason this research has focused on the influence of different substrates used mainly in Romania, for the rooting of cuttings shoot of *Abutilon hybridum*, with two variants: segment respectively peak shoot cuttings.
Also in the present research we try to identify an applicable statistic indicator which can quantify the experimental results. The intention is to use this type of indicator in other studies too, to check it viability as a rule as too in other ornamental species.

MATERIALS AND METHODS

The research was conducted in greenhouses of the Hortinvest Center of U.A.S.V.M. Bucharest. Long shoots were harvested from the mother plant in didactic collection. It had been made two types of cuttings of shoot: the peak section and - a number of 2-3 nodes each. Cuttings have different lengths due to the fact that the distance between the nodes on the shoot is different, the larger the smaller the base and the top, this being the average of 6.08 cm and 9.17 cm cutting the section for cutting the edge. After the taking the cuttings were then differently conditioned, the leaves from lower node were removed to root, and the other leaves from the upper nodes have been shortened by nearly half, to have a smaller leaf area with a less intense process of leaves transpiration.
It had been used 10 cuttings in each category for each substrate individually. Substrates for rooting cuttings were peat, perlite, river sand, and a mix of 1: 1 ratio between peat and sand.
Once fashioned, the cuttings were distributed properly in prepared substrates rooting, in alveolar plates.
Distribution and organization of these are shown in Table 1.

Table 1. The experimental variants

Experimental Variant	Substrate	Shoot cuttings		Total
		Segment	Peak	
V_1	Peat	10	10	20
V_2	Sand	10	10	20
V_3	Perlite	10	10	20
V_4	Peat+Sand	10	10	20
Total		50	50	100
Shoots medium lenght (cm)		6.08	9.17	

Throughout the period of the cuttings rooting it have intervened by applying a complex of specific care works for directing the micro climate factors.

RESULTS AND DISCUSSIONS

The rootedness process for *Abutilon hybridum* cuttings took place quite quickly, so after approximately 35 days after placing the cuttings in the rooting substrates, the roots were appeared.
The number of new plants (rooted cuttings) obtained greatly varies depending on the substrate used. Also, as occurs rooting process and we appear vegetative growth, so the terminal bud cuttings of new shoots started. Data on the number of rooting cuttings were obtained as summarized in Table 2.

Table 2. The percentage of rooting of the *Abutilon hybridum* cuttings depending on the cuttings type

Cuttings type	Rooting substrates			
	Peat	Sand	Perlite	Peat+Sand
Shoot segment	9	4	8	8
Shoot peak	8	6	10	9
Total	17	10	18	17
Rooting percent (%)	85	50	90	85

As can be seen, the largest percentage of rooting was registered in the cuttings placed on the substrate formed in perlite (90%), followed in descending order of results, on peat and peat + sand mixture (85%). The lowest yield was obtained from the rooting cuttings in sand (50%) (Figure 1).

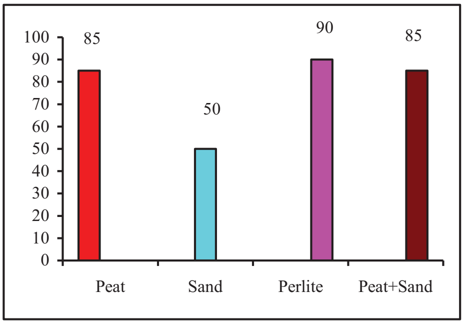


Figure 1. The percentage of rooting the *Abutilon hybridum* cuttings, depending on the substrate

Most favorable substrates for rooting cuttings peak shoot are the species *Abutilon hybridum*, the compose perlite mixture of peat + sand respectively; most unfavorable for this category is sand.

Most favorable substrates for rootedness process on the segment cuttings are peat and peat + shoot sand; most unfavorable for this category is sandy substrate.
Table 3 shows the experimental data presented averages of rootedness process, the ratio of the total number of rooted cuttings and total cuttings.

Table 3. The rooting percentage of *Abutilon hybridum* plants, depending on the cuttings type

Cuttings type	Number of cuttings placed on rooting substate	Rooted cuttings	Rooting percent %
Shoot segment	50	35	35
Shoot peak	50	40	40
Total	100	75	75

Thus, it can be seen that rooting was higher in cuttings from shoot peak (40%) compared with the segment one's (35%), this phenomenon could be explained by the fact that the cuttings in the first category have already top growth format, "energy" so saved can be channeled into a much consistencies of rootedness process.
This difference between the two types of cuttings, although not very significant lead to the idea that shoot tip cuttings have greater vigor and greater seating capacity than those obtained from the rooting sections shoots.
Results on root length obtained in the rootedness process are presented in Table 4.

Table 4. The length of the roots for the new plants obtained from cuttings

Rooting substrates	Roots lenght (cm)	
	Shoot segment cuttings	Shoot peak cuttings
Peat	7	9
Sand	16.45	16.02
Perlite	9.2	8.6
Peat+Sand	5.9	6.7

In the case of segment cuttings root it can be seen that evolution was largely uniform, excelling in the sand (16.45 cm), then descending in perlite (9.2 cm), peat (7 cm) and peat + sand mixture (5.9 cm).
The length of the roots where the plants from cuttings shoot peak was also influenced by characteristics of rooting substrates.
The results were recorded at a maximum of 16.02 cm in length, formed in the roots for the sand to a minimum recorded in the mixture of peat and sand (6.7 cm).
For the other two substrates were intermediate values of 9 cm length of roots (peat) or 8.6 cm (perlite) (figure 2).
For statistical analysis there was defined an indicator that reflects the two types of measurements: rooting percentage (%) and the average length of root cuttings (avg) for each type of substrate.

Figure 2. *Abutilon hybridum* new plants obtained from cuttings rooted in perlite

We normalized the data by minimax method (Myatt Johnson, 2014). Using these notations:
% T - segment cuttings rooting percentage;
% PS - peak cuttings rooting percentage;
avgT - the average length of roots on segment cuttings;
avgPS - the average length of roots on peak cuttings.
It was defined a first formula for the indicator ITPS (I = Rooting, T = Segment, P = Peak, S = Shoot) as follows:

$$ITPS = \frac{\%T + \%PS + avgT + avgPS}{4} \quad \text{(A)}$$

For the data about the rooting percentage for each substrate type we obtained an insignificant correlation ($r = 0.7$; $p = 0.23 > 0.05$) between the segment cuttings and the peak ones, while for the data obtained from the average length of roots for each substrate there were obtained a significant correlation ($r = 0.96$; $p = 0.04 < 0.05$) so it can remove the term avgPS in the formula (A) (because it correlates with avgT) and thus the index can be given by:

$$ITPS = \frac{\%T + \%PS + 2avgT}{4} \quad \text{(B)}$$

Another important aspect in defining this indicator is studying its consistency. For this we used a statistic instrument generally used as a measure of internal consistency, Alpha Cronbach's coefficient (α) (Cronbach, 1951):

$$\alpha = \frac{K}{K-1}\left(1 - \frac{\sum_{i=1}^{K} \sigma_{Y_i}^2}{\sigma_X^2}\right)$$

where K is the number of substrates, σ^2_X is the variance for all data and σ^2_{Yi} is the variance for each substrate. Since we obtain α = 0.79 for formula (A) and α = 0.81 for formula (B) we conclude we have assured the internal consistency of the indicator.

Analyzing the values of the indicator ITPS we observe that the best results were obtained in embodiments where the substrate was represented by perlite (0.63), peat (0.53) and sand (0.50). The lowest values of ITPS have been recorded in the cases in which peat was mixed with the sand (Figure 3).

In conclusion we can say ITPS is a reliable indicator who can help identify the optimal type of substrate for rooting cuttings. We recommend the use of the formula (A). Formula (B) is useful when measuring the roots of the peak shoot cuttings is more difficult. We used this indicator for substrates in which we had the proportion of 1:1 (peat + sand). ITPS indicator can also help us identify the optimal substrate when using substrates in different proportions.

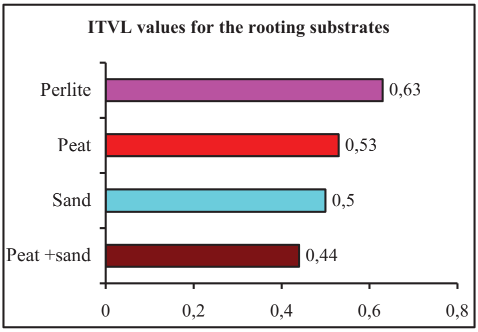


Figure 3. Values of ITPS for diferrent substrates

Vigorous growth of the roots in the variants where the substrate rooting was sand and perlite is explained by the influence of the particle size, texture slight growth stimulating cuttings root, covering both the section and those leading the shoot.

So for perlite it been obtained the best score of the index, while the lowest score was recorded substrate peat + sand. This indicator can be improved by the addition of costs incurred related factors (e.g. price of each substrate).

CONCLUSIONS

Following the experience of made rooting cuttings it been recommended, for peak shoot for Abutilon species, substrates composed of perlite, peat + sand and peat. Cuttings made from shoots segement section had a higher percentage of rooting for the substrate composed of peat and peat + sand. For both types of substrate cuttings rooting worst result was sand.

Root length obtained was highlighted in the case of the substrate consists of sand, for both types of cuttings. The mixture of peat + sand gave the lowest values for the length of root cuttings and the shoot tip cuttings section.

We consider it appropriate to continue the experience with the same components in mixtures with varying proportions and with other local substrates.

REFERENCES

Ahmed M. Eed, 2016. Effect Of Various Potting Media On Percent Survival And Growth Of Jojoba (Simmondsia chinensis) Rooted Cuttings, Int.J. Curr. Microbiol. App.Sci. 5(9), 454-461.

Burzo I., Delian E., Dobrescu A., Voican V., Bădulescu L., 2004. Fiziologia plantelor de cultură, vol.1, Ed CERES, București, 345.

Cronbach L.J., 1951. Coefficient alpha and the internal structure of tests. Psychometrika. 16 (3), 297–334.

Currey C.J., Hutchinson V.A, Lopez R.G., 2012. Growth, Morphology And Quality Of Rooted Cuttings Of Several Herbaceous Annual Bedding Plants Are Influenced By Photosyntetic Daily Light Integral During Root Development – Hortscience, 47(1), 25-30.

Davidescu V., Madjar R., Costea G., Stanica F., Carețu G., 2001. Substraturi de cultură, Editura Ceres, Bucuresti, 16:30

Fanghua Niu, Donglin Zhang, J. Ruter, Zhihui Li, 2013. Propagation And Cross Compatibility Of Abutilon, Ishs Acta Horticulturae 1055: Proceedings of the International Plant Propagators Society,

Kim J., van Iersel M.W., 2009. Daily Water Use Of Abutilon And Lantana At Various Substrate Water Contents. Proc. Southern Nursery Assn. Res. Conf. 54:12–16.

Myatt G.J, Johnson W.P., 2014. Making Sense Of Data I: A Practical Guide To Exploratory Data Analysis And Data Mining, John Wiley and Sons Publishing House, New Jersey.

Șelaru E., 2005. Plante de apartament, Ed. Ceres, București, Ediția II, 59:66.

Șelaru E. 1995. Culturi de seră pentru flori tăiate, Ed. Ceres, București, Ediția I, 48:56.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDY ON RESULTS OBTAINED BY DIFFERENT RESEARCHERS ON *IN VITRO* PROPAGATION OF HERBACEOUS PEONY

George Nicolae CAZAN, Florin TOMA

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: cazan.nic@gmail.com

Abstract

These researches highlights the advances made on various aspects of herbaceous peony (Paeonia lactiflora Pall.) tissue culture, including the in vitro culture of underground buds, leaves and petioles, the induction of cluster buds, and callus induction. Tissue culture has the ability of speeding up the rate of propagation, reducing breeding time and meeting the needs of mass production. Problems that are currently being faced in herbaceous peony tissue culture are highlighted and possible viable solutions are provided. More research is needed to make mass production of peony more commercially successful. Optimization of procedures are necessary, from selection of explants, decontamination, screening of medium, application of plant growth regulators (PGRs), induction of callus and shoots, subculture, rooting, and to final transplanting. The major goal of these studies is to evaluate shoot induction ability of peony explants and PGRs. The development of micropropagation methods for peonies is necessary to not only overcome this problem but also accelerate peony breeding progress. Tissue culture is one of the most effective approaches for rapid propagation of plants and is also providing a new approach for plant breeding in crops, ornamentals, fruits and vegetables. Effect of Gelling Agents on Tissue Culture of Herbaceous Peony. Comparison of Basal Medium in Tissue Culture of Herbaceous Peony. Effect of Pretreatment of Underground Buds on Tissue Culture of Herbaceous Peony. Investigation of Callus and Shoot Induction Ability of Flower Tissue. Effect of PPM on Decontamination and Rescue of Explants. Root Induction and Transplanting of Herbaceous Peony In Vitro.

Key words: *herbaceous peony, Paeonia lactiflora* Pall.*, tissue culture, in vitro propagation.*

INTRODUCTION

Peonies can be propagated by division, cutting, grafting, and layering to obtain true-to-type plants.

There are multiple traditional choices for propagation of peony. However, the limited number produced by these traditional methods can not meet the increasing demands in the market, especially for a quick release of a new cultivar and massive production of a specified variety. The development of micropropagation methods for peonies is necessary to not only overcome this problem but also accelerate peony breeding progress.

The field of plant tissue culture is based on the premise that plants can be separated into their component parts (organs, tissue, or cells), which can be manipulated in vitro and then grown back to complete plants. The first successful plant tissue and cell culture was accomplished by Gottlied Haberlandt near the turn of the 20th century when he reported the culture of leaf mesophyll tissue and hair cells (Steward, 1968; Krikorian and Berquam, 1969).

Micropropagation of peony began in the middle 1960s. During the last 40 years, much research on micropropagation of peony has been conducted with plants successfully produced from tissue culture labs. Planteck Biotechnologies Inc., a company based in Quebec, Canada, produced mass numbers of herbaceous Itoh peonies and made the tissue cultured plants available in the market for the first time in 2006 (Whysall, 2006).

However, there is still much work to be done in order to make mass production of peony more commercially successful. Optimization of procedure for each stage or step should be studied in depth, including selection of explants, decontamination, screening of medium, application of plant growth regulators (PGRs), induction of callus and shoots, subculture, rooting, and finally transplanting.

In peony, callus was induced successfully for the first time by Yamada and Sinotô (1966) from petal culture of *P. japonica*. Large variation in chromosome numbers and characteristics within cells was observed during culture. Demoise (1967) and Demoise and Partanen (1969) induced callus from tree peony and investigated the effects of subculturing and physical conditions of culture on the mitotic cycle kinetics of a population of cells, particularly in relation to the degree of heteroploidy. Since then, many original studies on in vitro culture of peony have been reported. Several review papers have also been published. The first review on micropropagation of peony was published by Buchheim and Meyer (1992), where research on *in vitro* culture of peony before 1989 on has been mostly discussed. A summary of in vitro studies conducted on several species of *Paeonia* was included in a table consisting of plant species, inoculum, medium, growth response, and reference. This review is useful for a researcher working on peony tissue culture, although some medium formulations have not been cited correctly. The second review on in vitro culture peony was written by Gabryszewska (2004). The regeneration ability of different organs of both herbaceous and tree peony was reported in this paper. The role of exogenous and endogenous PGRs in differentiation and growth of shoots, roots, and somatic embryos was also discussed.

In 2007, Beruto and Curir summarized tissue culture of tree peony under a book chapter title, '*In vitro culture of tree peony through axillary budding*', which included most of the results published between 1969 and 2004. The review was well organized by introduction, experimental protocol consisting of stages of tissue culture, and conclusion. During this time, five other review papers on peony tissue culture were published by Chinese researchers (Buchheim and Meyer, 1992; Gabryszewska, 2004; Li and Luo, 2004; Li et al., 2006a; Jia et al., 2006b; Meng et al., 2007, Beruto and Curir, 2007 2007; Zhao et al., 2007). However, each of these papers is only 2 to 4 pages long and did not cover as much as information of previous work on micropropagation of peony. With the fast development of internet and database construction in the world and the benefit of language translation software, it is becoming much easier to access and understand the original research resources published by non-native languages.

More research is needed to make mass production of peony more commercially successful. Optimization of procedures are necessary, from selection of explants, decontamination, screening of medium, application of plant growth regulators (PGRs), induction of callus and shoots, subculture, rooting, and to final transplanting. The major goal of this study is to evaluate shoot induction ability of peony explants and PGRs.

EFFECT OF GELLING AGENTS ON TISSUE CULTURE OF HERBACEOUS PEONY

During this researches made in 2007 Torres et al., used as material and method the follouing:

Four types of agar products were used to compare responses of nodal stem explants of 'Yang Fei Chu Yu' ('YFCY') and 'Xi Shi Fen' ('XSF') in ½ MS + 1 mg l-1 TDZ medium with (1) A111 (Phytotechlab) 5 g l^{-1}; (2) A296 (Phytotechlab) 6 g l^{-1}; (3) A133 AgagellamTM (Phytotechlab) 4 g l^{-1}; and (4) A20020 (high gel strength) 5 g l^{-1} according to the recommended rates.

After this researches made in 2007 Torres et al., obtained the follouing result and discutions:

Explants responded to the types of agar gelling agents differently. Generally A111 (5 g l^{-1}) and A133 (4 g l^{-1}) were much better than A296 (6 g l^{-1}) and A20020 (5 g l^{-1}) for all indexes. Response was also genotype dependent. A133 and A111 significantly increased explant growth of 'YFCY' as compared to A296 and A20020 but this effect did not occur in 'XSF'. Similar results were noted on induction of callus and shoots. The highest rates of shoot initiation and callus production were seen on explants with treatments of A111 and A133, respectively, in 'YFCY', but no significant difference was observed in 'XSF'. For both cultivars, more phenolic compounds exuded into media with A296 and A20020, and explant color looked abnormal. Agar has long been used to solidify media for plant tissue culture. There are lots of brands of agar gelling agents.

The type of agar or other gelling agents significantly influences plant growth in tissue culture. Differences of results are even seen in the same product made at different times or locations (Torres et al., 2007).

COMPARISON OF BASAL MEDIUM IN TISSUE CULTURE OF HERBACEOUS PEONY

During this researches made in 2008 Daike Tian used as material and method the follouing

The choice of type of basal medium is important for tissue culture. MS, ½ MS and WPM have been mostly used in peony tissue culture for shoot induction. In this experiment, effects of MS (1/4, half, full strength) and WPM (half, full strength) medium with 1 mg l^{-1} TDZ + 0.1 mg l^{-1} BA + agar (A111, 4 g l^{-1}) was evaluated on callus and shoot induction of nodal stems from three herbaceous cultivars: 'Bin Shan' ('BS'), 'Fen Ling Hong Zhu' ('FLHZ') and 'Yang Fei Chu Yu' ('YFCY').

After this researches made in 2008 Daike Tian obtained the follouing result and discutions

The choice of type of basal medium is important for tissue culture. MS, ½ MS and WPM have been mostly used in peony tissue culture for shoot induction. Explants generally performed best in full strength medium MS followed by half strength MS, and worst in half strength WPM. Nodal stems of all three cultivars remained green in full strength MS medium, while explants turned pink and showed slight abnormalities in other MS media. Explants cultured in WPM turned pink, then red over time. MS was the most favorable medium for explant growth of 'BS'. Explants cultured in ½ WPM had the least increase in growth for all three varieties. Browning was more visual in WPM than MS. MS medium was more favorable than WPM for callus induction but results of shoot induction were complicated. Explants cultured on full strength WPM produced the highest shoot initiation rates for 'BS' and 'FLHZ' but the lowest rate for 'YFCY'. Explants of 'BS' generated the lowest shooting rate on half strength WPM while explants of 'FLHZ' produced the lowest shooting rate in ½ MS. Compared with the data collected on 24 and 40 days after inoculation, the difference in the initiation rate among basal medium types was consistent.

EFFECT OF PRETREATMENT OF UNDERGROUND BUDS ON TISSUE CULTURE OF HERBACEOUS PEONY.

During this researches made in 2008 Daike Tian used as material and method the follouing:

Responses of underground buds to three pretreatments were evaluated in tissue culture of two varieties of herbaceous peony. Underground buds (Figure 1) from containerized stock plants of 'Da Fu Gui' ('DFG') were treated in three ways: (1) plants remained untreated; (2) plants were treated in cooler for 20 d beginning Nov. 20, 2006; and (3) plants from outside were washed by tap water and repotted in container with perlite, then directly moved to greenhouse to break dormancy and force growth.

Figure 1. Underground buds of herbaceous peony, (after Daike Tian, 2008)

In this treatment, the stock plants were also rinsed with 1% Zerotol for two times, once per week, to decrease contamination. After surface sterilization of 20 sec in ethanol (75%) following by 25 min in 10% Chlorox bleach, the buds (tips) were inoculated in test tubes with medium ½ MS + 1 mg l^{-1} BA + 0.1 mg l^{-1} GA3 or ½ MS + 0.1 mg l^{-1} BA + 0.1mg l^{-1} GA3 on Dec 10, 2007. Shoot multiplication medium was ½ MS + 0.1 mg l^{-1} BA + 0.1 mg l^{-1} TDZ + 0.1 mg l^{-1} GA3.

After this researches made in 2008 Daike Tian obtained the following result and discutions

A previous experiment produced 100% contamination of 'DFG' and 'Xi Shi Fen' ('XSF') dormant underground buds with a sterilization treatment of 10 sec in ethanol (75%) following by 10-20 min in 10% Chlorox bleach. After 15 d of culture, no callus formed and buds did not grow. In this experiment, contamination and phenolic exudation 281 were visual within one day of culture and increased over the time of the 15-d experiment. All explants (100%) treated in greenhouse were contaminated within 3 d of culture. The cooler treated explants performed better. Only 2 of 16 explants were contaminated within three days although contamination rate went up to 75% after 15 d culture. More explants were contaminated by bacteria than fungi. Contamination with bacteria also occurred earlier. There was significant difference in fungi caused infection among treatments. At the end of experiment, only one bud from the outside treatment and 4 buds from the cooler treatment were sterile for use of shoot induction after transfer. Before culture, buds treated in the cooler for 20 d were already slightly elongated (dormant release) and looked better in quality than both outside and greenhouse treated buds. After inoculation these buds grew very fast and main shoots as well as some lateral shoots elongated (Figure 2). These elongated shoots could be cut into nodal sections and transferred to fresh medium for shoot multiplication. Buds from the outside also elongated but did not grow as fast as the cooler treated buds. The shoots were much shorter than those generated from the cooler treated buds (Figure 2). The greenhouse perlite medium treated buds performed worst and all nearly stopped growing. These buds finally died and no axillary shoots generated. For all treated buds, no callus formed in the early stages. Only very little green callus was induced at the cutting side of buds after more than 15 d of culture. A more effective sterilization approach must be developed. Dormant buds were not effective for shoot induction.

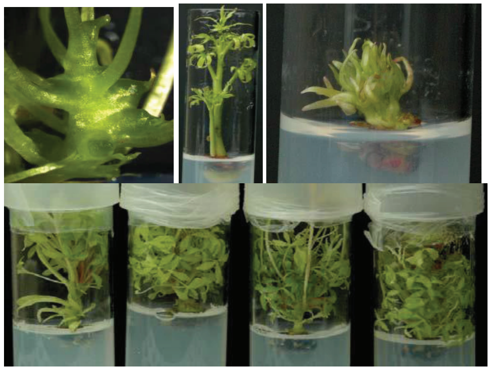


Figure. 2. After culture in ½ MS + 1 mg l^{-} BA + 0.1 mg l^{-} GA3, the cooler treated buds (left) grew fast and produced elongated shoots (main and lateral). After cutting of shoots and subculture in ½ MS + 0.1 mg l^{-} BA + 0.1 mg l^{-} TDZ + 0.1 mg l^{-} GA3, the number of shoots was multiplied, each bud eye region formed one to several new shoots (middle). While bud explants from outside stock plants (right) grew slow and shoot stem did not elongated much (after Daike Tian, 2008)

If the buds were treated in cooler, contamination decreased significantly. Shoot or bud explant could readily grow and axillary shoots were easily induced after bud dormancy was broken.

INVESTIGATION OF CALLUS AND SHOOT INDUCTION ABILITY OF TISSUE

During this researches made in 2008 Daike Tian used as material and method the following

Callus and shoot induction ability of 'Da Fu Gui' ('DFG') and 'Paula Fay' ('PF') were studied in this experiment using petals, anthers, filaments, and pistils as explants from the tight flower buds (TFB), one week before blooming, newly opening flowers (OF) with a small mouth or the fully open flowers (FOF). The petals of TFB, OF and FOF were from 'DFG', and the petals, anthers and filaments of TFB were from 'PF'. Three media were tested: A: ½ MS (PGR-free); B: ½ MS + 1 mg l^{-1} TDZ; and C: ½ MS + 1 mg l^{-1} TDZ + 0.1 mg l^{-1} GA3.

After this researches made in 2008 Daike Tian obtained the following result and discutions

After 14 d of culture, contamination occurred on the petals but not on either anthers or filaments. Petals from FOF had higher contamination rate than those from TFD and OF in 'DFG'. Browning was obvious in all treatments and it was more severe on the petals of FOF followed by OF in 'DFG'. Petals from TFB had the least browning problem. However, in 'PF' nearly all of the petals from TFB turned brown. The least browning rate was seen on anther culture of this cultivar. No difference in browning was found among PGR treatments. There were large differences in growth of explants among types of petals. Petal sections from both TFD and OF grew fast, whereas those from FOF grew slow and lost their original color within two days of culture. Callus generated very slowly and no callus formed within 15 d. Following 30 d of culture, very little callus was induced from the base cutting side of the petals from TFB and OF in both B and C media.

EFFECT OF PPM ON DECONTAMINATION AND RESCUE OF EXPLANTS

During this researches made in 2008 Daike Tian used as material and method the follouing

Effect of PPM on decontamination of nodal stem segments from 'Da Fu Gui' (treated 3 months in the cooler) and rescue of contaminated explants was investigated in media with ½ MS + 1 mg l^{-1} BA + 0.2 mg l^{-1} GA3 with supplement of 0, 0.05, 0.1, and 0.2% (v/v) of PPM, respectively. The explants were surface sterilized with a 12–15 sec quick soak in 70% Ethanol followed by 20 min in 10% Bleach. For rescue of contaminated explants, 12 of bacteria contaminated explants were cleaned by a soft brush under running tap water following by a 12 min soak in 50% PPM and then were inoculated on fresh medium.

After this researches made in 2008 Daike Tian obtained the follouing result and discutions

PPM had effect on minimizing contamination of explants and the contamination rate decreased in 0.2% PPM-treated medium. However, lower concentrations (<0.2%, v/v) of PPM only delayed occurrence of contamination. No difference was observed in browning, the callus and shoot induction rate, and shoot growth of posttransferred explants between PPM treatment and the control explants. This indicated application of PPM at low level in medium had no side effect on tissue culture of peony.

ROOT INDUCTION AND TRANSPLANTING OF HERBACEOUS PEONY *IN VITRO*

During this researches made in 2008 Daike Tian used as material and method the follouing

Several trials on root induction were conducted on vitroshoots from the varieties: 'Xi Shi Fen', 'Da Fu Gui', and 'Cytherea'. The treatments included following variables: light and darkness; liquid (paper bridge method, Hosoki et al., 1989), half solid and solid ½ MS 285 medium; activated charcoal (AC); temperature; and IBA with different concentrations or a quick soak in high concentration of IBA (10 mg l^{-1}).

After this researches made in 2008 Daike Tian obtained the follouing result and discutions

Following 7–10 d of culture in rooting medium, all vitroshoots generated callus. Callus even formed sometimes on the petioles.

Plantlets rooted following about 20 d of culture and up to 20 roots developed on a shoot. Roots grew up to 3 cm after 45 d of shoot inoculation. The vitroshoots grew very fast in semi-solid rooting medium possibly benefiting from an easy absorption of nutrients (Figure 3). AC treatment increased the shoot length but not significantly. IBA shock treatment (10 mg l^{-1}) did not influence growth of *in vitro* shoots on rooting medium. IBA at 1 mg l^{-1} in a continuous treatment was too strong for small younger vitroshoots and caused their petioles to calluse. It remains not clear how long vitroshoots should be treated in medium with high concentration IBA before transfer to root growth medium with either lower concentration of IBA or no IBA. High-quality shoots are the basic requirement to obtain high rate of rooting. Several studies have reported treatment of darkness and chilling was beneficial for rooting of peony in vitro (Kunneman and Albers, 1989;

Habib and Donnelly, 2001; Beruto et al., 2004; Chen 2005).

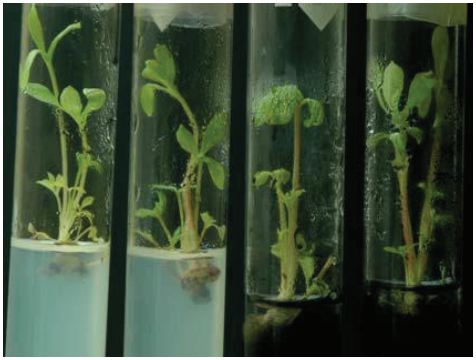


Figure 3. Shoots grew fast in half-solid medium and there was no difference in shoot length between treatments (with or without IBA shock, AC), (after Daike Tian, 2008)

Roots were successfully induced from plantlets in solid medium (Figure 4) or using paper bridge method (Figure 5) in some cases.

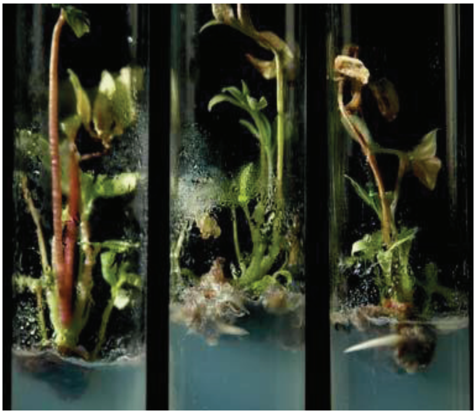


Figure. 4. Roots were only induced on the shoots with a 10-min pretreatment in high concentration of IBA (10 mg l^{-}) before inoculation on medium with ½ MS + 1 mg l^{-} IBA ± 5% AC (w/v) (after Daike Tian, 2008)

The present experiment showed that the shoots with a 40-d darkness treatment at 25ºC nearly stopped growth and completely died later. The shoots with a 40-d darkness treatment at 10ºC survived with chlorotic tissue. However, no roots were obtained in both treatments. For both of the rooted and non-rooted shoots, the earlier developed leaves showed obvious necrosis after more than two months culture but the later formed young leaves remained healthy. It was not clear if necrosis problem could be avoided or minimized by an increase of transfer frequency. Limited number of shoots with roots or root primordia was transplanted to jars with non-sterile peat moss. These vitroplants grew in the first week but quickly died because of infection with fungi (Figure 6).

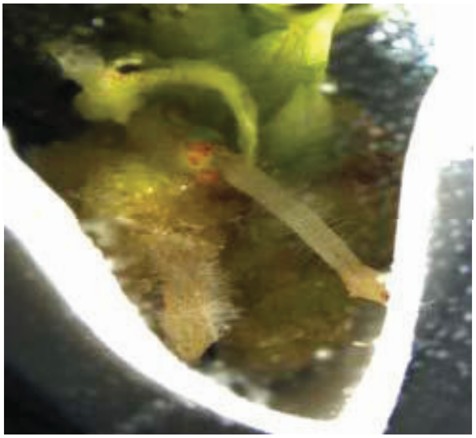


Figure 5. Roots were induced from vitroshoots of 'Da Fu Gui' by paper bridge (white material) method with liquid medium: ½ MS + 1 mg l^{-1} IBA (after Daike Tian, 2008)

Figure. 6. After transplanting, rooted shoots grew in the first week but then died with contamination of fungi or rot of roots and stem (after Daike Tian, 2008)

CONCLUSIONS

As a result of their researches made in 2007 Torres et al., reached follouing conclusion

The type of agar or other gelling agents significantly influences plant growth in tissue culture. Differences of results are even seen in the same product made at different times or locations.

As a result of their researches made in 2008 Daike Tian, reached follouing conclusions

1. The highest shoot length was observed in explants of 'BS' cultured on 1/2 MS and 'FLHZ' on MS. For these two cultivars, shoot growth of explants performed worst on ½ WPM. Shoot growth generally increased from ¼, ½, to 1 MS. Quality of generated shoots was better in MS than WPM medium.
2. It has been widely reported that underground buds of herbaceous peony are difficult to disinfect compared with aerial tissues. Response of underground buds was different in tissue culture between stages of growth or when different pretreatments are used. . Contamination was a big problem with the use of underground buds as explants in tissue culture of herbaceous peony.
3. No callus was seen in A (PGR-free) medium for petals. Anthers and filaments also did not produce callus. Results indicated 1 mg l^{-1} TDZ could induce callus from petal culture. However the induction rate as well as the amount of callus was very low. There was no difference in callus induction between B and C media. It showed that GA3 at 0.1 mg l^{-1} concentration had no effect on induction and production of callus compared with the control. No adventitious shoots developed.
4. Contamination of the rescued contaminated explants significantly decreased following PPM treatment but black phenolics exuded quickly to medium from explants. Browning was a big problem which quickly caused death of plant material. Therefore, it was not a viable option to rescue contaminated peony explants using PPM even though successful in decontamination.
5. Treatment with a spray of 1% Hydrogen peroxide (H2O2) did kill fungi but could not save the plantlets. Four plantlets transplanted to sterile peat-moss mix died quickly with rot of roots and stem base.

REFERENCES

An B.Y., 2005. Studies on the establishment of in vitro regeneration system of *Paeonia suffruticosa* Andr. Northeast For Univ, MS Thesis.

Beruto M, Lanteri L, Portogallo C., 2004. Micropropagation of tree peony (*Paeonia suffruticosa*). Plant Cell Tissue Organ Cult 79:249-255.

Beruto, M., Curir P., 2007. In vitro culture of tree peony through axillary budding, Protocols for micropropagation of woody trees and fruits. Springer, 477-497.

Buchheim J.A.T., Meyer M.M.Jr., 1992. Micropropagation of peony (*Paeonia* spp.). In: Bajaj, Y.P.S. (Ed.). High-tech and micropropagation IV. Biotechnology in Agriculture nd Forestry V 20:269-285. Springer-Verlag. Berlin Heidelberg.

Chen X.L., 2005. The preliminary research on tissue culture of *Paeonia suffructicosa*. Henan Agr. Univ., MS Thesis.

Daike T., 2008. Container Production and Post-Harvest Handling of Lotus (Nelumbo) and Micropropagation of Herbaceus Peony, A Dissertation Submitted to the Graduate Faculty of Auburn University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy, Auburn, Alabama.

Demoise C.F., 1967. Effects of subculture and physical condition of media on a plant issue culture (*Paeonia suffruticosa*) Univ. Pittsburgh, PhD Diss. Univ. Microfilms 68-4431.

Gabryszewska E., 2004. Regeneration and growth of peony (*Paeonia* spp.) *in vitro*. Acta Agrobotanica 57:5-19.

Habib A, Donnelly DJ, D'Aoust L., 2001. Micropropagation of herbaceous peony. Amer. Peony Soc Bull 319:19-24.

Hosoki T, Ando M, Kubara T, Hamada M, Itami M., 1989. *In vitro* propagation of herbaceous peony (*Paeonia lactiflora* Pall.) by a longitudinal shoot-split method. Plant Cell Rpt 8:243-246.

Jia, W.Q., H.C. Liu, and L.F. Yao. 2006b. Research development of *Paeonia* spp. tissue ulture. J. Anhui Agr. Sci. 34:2725-2727, 2729.

Kunneman BPAM, Albers MRJ.. 1989. Tissue culture of peony is not yet producing plants. Bloembollencultuur 100:16-17.

Krikorian, A.D., Berquam D.L., 1969. Plant cell and tissue culture ─ the role of Haberlandt. Bot. Rev. 35:59-88.

Lang Y.T, Luo X.F., 2007. The research on callus induction and browning prevention of peony. J Henan For Sci Technol 27:4-6.

Li Y.M., Luo X.F., 2004. Research development of in vitro breeding and fast propagation of *Paeonia* spp. J. Southwest For. Colloge 24:70-73.

Li Z.F., Hu Y.H., Liu Q.H., Wang J., 2006. Research advance in Chinese peony tissue culture. J. Anhui Agri. Sci. 34:2998-2999, 3001.

Meng J., Han J., Zu E.P., 2007. Advances in tissue culture of *Paeonia suffruticosa*. Northern Hort. (1):153-154.

Orlikowska T, Marasek A, Kucharska D., 1998. Regeneration of *Paeonia mlokosewitschii* Lom. and *P. tenuifolia* L. *in vitro* from different explants. Acta Soc Bot Poloniae 67:223- 227.

Steward F.C., 1968. Growth and organization in plants. Addison-Wesley Publishing, Reading, MA.

Torres K.C., Seckinger G.R., Burdick E.D., 2007. A review of gelling agents used for plant tissue culture: their sources and characteristics. *In vitro* Cell. Dev. Biol. 43:S14 (Abstr.).

Yamada T., Sinoto Y., 1966. On the variation of chromosome in the cultured cells of *Paeonia japonica*. Jpn. J. Genet. 41:488 (Abstr.).

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE INFLUENCE OF FERTILIZATION REGIME ON MORPHOLOGICAL, ANATOMICAL AND PRODUCTIVE CHARACTERISTICS OF *PELARGONIUM CITROSUM* PLANTS

Florin TOMA, Mihaela Ioana GEORGESCU, Sorina PETRA, Diana ZAMFIR-VÂȘCĂ, Elena SĂVULESCU, Cristina Rodica MĂNESCU, Vasilica LUCHIAN, Vlad POPA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding authors email: florintoma@hotmail.com, mihaelaigeorgescu@yahoo.com

Abstract

Pelargonium citrosum is much appreciated both due to increased rich and very beautiful appearance of the leaves and the strong scent that exudes the whole plant. It drew this perfume plant called mosquito plant. Recent research has shown, however, that this name is not justified in removing the effect of plant mosquito void. It is clear however that this fragrance because Pelargonium citrosum plants are used not only for decoration but also as a raw material in the food, cosmetic and pharmaceutical. Getting rich growth of plants and compact to serve both decorative purposes and as a raw material in the industries mentioned above was the objective of our research. The plants were obtained by cutting and then were applied to four different types of fertilizers. Plant growth and development was pursued both by macroscopic biometric measurements (diameter, height and weight of plant, no. of leaves) and microscopic analysis of tissue by incorporation of leaf lamina and petiole. The results of this research show that the highest values of the parameters determined (both macroscopic and microscopic ones) were recorded in the variants which fertilization was applied twice a month and nitrogen have slightly exceeded phosphorus and potassium.

Key words*: fertilizer, plant growth, limb leaf, petiole leaf, Pelargonium citrosum.*

INTRODUCTION

The *Pelargonium citrosum* plant is the subject of numerous research conducted around the globe (Matsuda and al., 1996; Nianqiong and al., 2006; Liu Yu-mei and al., 2009).

Many of the research is aimed at testing the alleged repellent properties of mosquito plants (Matsuda and al., 1996) or to in vitro plant multiplication (Gill R. and al., 1992; Wei HP, Tan JY, Chen S, 2005; Zhou J. and al., 2007). For this reason, it is commonly known as the mosquito plant. Plants of *Pelargonium citrosum* are grown, on a large scale, for their aesthetic, medicinal and culinary properties (Toma, 2009).

Our research has focused precisely on these aesthetic, medicinal and culinary properties of *Pelargonium citrosum* plants.

Through our research, we wanted to obtain a massive plant mass that would be of benefit both from the ornamental point of view and from the quantity of raw material used in the pharmaceutical and culinary industry (Toma, 2009).

MATERIALS AND METHODS

Macroscopic observations

To study the influence of the NPK on plant growth we used four types of fertilizer with different reports on nitrogen, phosphorus and potassium (Toma, 2009). Thus, for the first fertilizing plant (V0), a fertilizer with a NPK ratio of 16:11:10 was used, applied to the planting of the rootstocks. For plants of the second fertilizer variant (V1), a fertilizer with a NPK ratio of 10: 5: 6 was applied at one month interval. For plants of the third fertilization variant (V2), a fertilizer with a NPK ratio of 7: 4: 5 was applied at two weeks. Four fertilizer with a NPK ratio of 4: 5: 6 was applied weekly to the 4th fertilizer plants (V 3). At the end of the six months of different fertilization programs we weighed the plants for each of the fertilization variants.

Also, we registered the total number of leaves by plants.

Microscopic observations

In order to observe the influence of fertilization variant on the internal structure of *Pelargonium* plants were analysed tissues constituents of the leaf, blade and petiole, in the cross-sections. For the blade, were measured both the epidermis and the cuticle layer and the mesophilic tissues. For the petiole were analysed the epidermis, parenchyma tissue and sclerenchyma, both the tissue and cell walls thickness. The cross-sections were clarified with a saturated solution of Chloral hydrate for 24 hours, washed 2-3 times with distilled water, acidified water with 1% acetic acid and again distilled water and stained with Alun-Carmine and Iodine Green to highlight the cell wall structure (Georgescu M.I. and al., 2001). Images were obtained with a digital camera (Panasonic DMC-LZ7) at an optical microscope (Optika DM-20).

RESULTS AND DISCUSSIONS

Macroscopic observations

The weighing of the plants after six months of application of the fertilization program revealed the following values of the weight of the plants: 85.81 g for the plants of variation V0; 67.09 g for V1 plants; 92.80 g for V2 plants; 81.88 g for V3 plants (Figures 1-5).

Figure 1: Weight of plants from variant V0

Figure 2: Weight of plants from variant V1

Figure 3: Weight of plants from variant V2

Figure 4: Weight of plants from variant V3

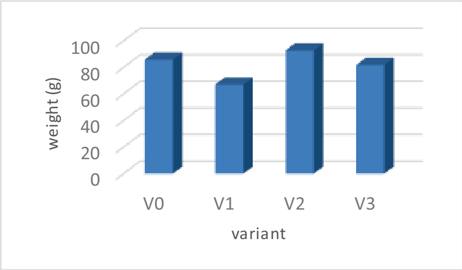


Figure 5: The variation of weight of plants

The number of leaves on the plant varied between 92, at V 1, with the NPK ratio of 10: 5: 6 and the monthly application frequency and 185 at V2 with the NPK ratio of 7: 4: 5 and the bi-monthly application frequency (figure 6).

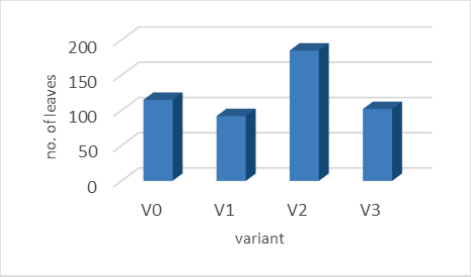


Figure 6: The variation of no. of leaves on the plants

Microscopic observations

The blade. The cross section of the blade shows the two epidermis, upper and lower, consisting of a single layer of cells protected by a cuticle.

On both surfaces are multicellular secretory hairs accompanied by bicellular protective hairs, only on upper epidermis. The mesophyll is bifacial type, with a single row of cells in the palisade tissue.

The highest value of the mesophyll thickness - 20.03 µ is recorded on variant V2, the mesophyll of the remaining variants having relatively similar size (17.00 - V3; 17 07 µ - V0, respectively 17, 53 - V1) (Table 1)

Cells' height of the lower epidermis is between 1.53 µ (variant V2) and 1, 40 µ (variant V3) (Table 1).

The measurement values of the upper epidermis ranges between 2.03 µ (variant V0) and 2.64 µ (variant V1) and of the cuticle between 0.37 µ (variant V2) and 0.51 µ (variant V0) (Table 1). There is a correlation between these values and the thickness of the mesophyll (Figures 7, 8).

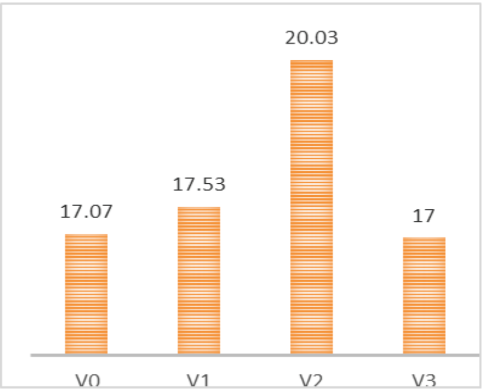


Figure 7: Mesophyll thickness (µ)

Towards the inside of the cross-sections there are collateral vascular bundles on one circle, limited outwards by an arch of sclerenchyma, linked to a mesarch sclerenchyma.

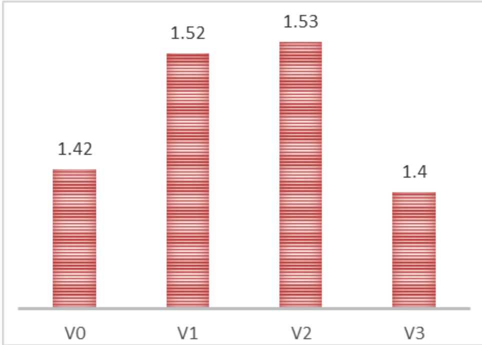


Figure 8: Lower epidermis thickness (µ)

A chlorenchyma is under the epidermis. The thickness of this parenchyma varies from 11.57 µ (variant V3) and 13.43 µ (variant V0) (Table 1). The height of the epidermal cells has values between 1.25 µ (variant V0) and1.31 µ (variant V2) (Table 1).

The petiole. The petiole's cross-section is circular; the epidermis, with a single cells layer, is covered with glandular and protective hairs on the abaxial side (Figure 9)

Sclerenchyma tissue and cell wall thickness showed higher values in the variants in which nitrogen is in small quantity, at V2 and V3 variants, to V0 and V1 variants (Table 1, Figure 9).

Figure 9: Variant V 2, petiole – cross-section
p – glandular hair; ep – epidermis; chl. – chlorenchyma; fc. – vascular bundles; schl. – schlerenchyma

Table 1: Morphometric values (µ) of the internal structure components of the leaf blade and petiole depending on the variant of fertilization

Var	NPK ratio	Lamina				Petiole				
		upper epidermis	cuticle	mesophyll	lower epidermis	epidermis	parenchyma	sclerenchyma bundles	mesarch sclerenchyma	wall's sclerenchyma cell
V0	16-11-10	2.03	0.51	17.07	1.42	1.25	13.43	3.05	3.04	0.29
V1	10-5-6	2.64	0.44	17.53	1.52	1.24	12.48	2.63	2.97	0.35
V2	7-4-5	2.43	0.37	20.03	1.53	1.31	12.23	4.02	3.51	0.41
V3	4-5-6	2.34	0.38	17.00	1.40	1.28	11.57	3.41	3.30	0.42

CONCLUSIONS

Bimonthly fertilization with fertilizer with a slightly nitrogen-friendly NPK gives rise to a higher weight of *Pelargonium citrosum* plants.
The beneficial effect of this variant of fertilization is also evidenced by the recording of higher morphometric values of the components of the internal structure of the foliage and petiole.

REFERENCES

Gill R, Gerrath J, Saxena PK, 1992. High-frequency direct embryogenesis in thin layer cultures of hybrid seed geranium (*Pelargonium*). Canadian Journal of Botany 71, 408-413.

Georgescu Mihaela Ioana, Savulescu Elena, Padure Ioana Marcela, Luchian Vasilica, 2001. Practical works on botany-plant morphology and anatomy. Ed. of USAMV Bucharest.

Liu Yu-mei and col., 2009. Research Advance in the Biological Characteristics and Cultivation Technologies of *Pelargonium* [J]; Journal of Anhui Agricultural Sciences; 2009-17.

Matsuda BM, Surgeoner GA, Heal JD, Tucker AO, Maciarello MJ (1996) Essential oil analysis and field evaluation of the citrosa plant "*Pelargonium citrosum*" as a repellent against populations of Aedes mosquitoes. Journal of the American Mosquito Control Association 12, 69-74.

Nianqiong Jiang Zhang Rui, Zheng Yizhi, Clonal Propagation of *Pelargonium citrosum* Vanleenii and Main Factors Affecting its Large-scale Production. Chinese Agricultural Science Bulletin 2006-09.

Toma F. Floriculture and Floral Art. Vol. I and III. Ed. InvelMultimedia Bucharet-Otopeni, 2009.

Wei HP, Tan JY, Chen S, 2005. Study on tissue culture and plant regeneration of *Pelargonium* × *citrosum* Vanleenii. Journal of Anqing Teachers' College 11, 50-53.

Zhou J. Yang Miao-xian, Xian Yuan-xiang, 2007. In Vitro Shoot Organogenesis from *Pelargonium* x *citrosum* Vanleenii Leaf and Petiole Explants. Floriculture and Ornamental Biotechnology, 2007, Global Science Books.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

IDENTIFICATION AND PRESERVATION OF CULTURAL AND LANDSCAPE IDENTITY – THE PLANE TREE PARK OF BUCHAREST

Elisabeta DOBRESCU[1], Sanda PETREDEANU[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania
[2]The National Bank of Romania, Bucharest, Romania

Corresponding author email: veradobrescu@gmail.com

Abstract

Urban green spaces are defined as city areas where complex interaction of environmental, human, socio-economic and cultural factors take place, that gives us a dynamic perspective, but with some stability, given by the customs and values of a society under transformation. The valuable urban landscape, both culturally and in terms of the quality of urban life, is a subject that can be approached among climate, social, and territorial changes attempt by the city in its evolution. Urban green spaces under increased real estate and social pressure are gradually diminished in terms of quality and value. This leads to focusing on elements that serve as memorial landmarks, heritage landscape that can define and characterize the evolution of a society. The secular trees, or those white aesthetic, historical, memorial and social value, are such cultural landmarks that require great attention, taking into account their value, doubled by the lack of a perspective to protect and preserve historical and landscape significance. In the current context, public or private green areas of Bucharest has a total of 110 specimens of protected trees, considered natural monuments, according to the List of protected trees, managed by the Romanian Academy. An urban green area less known but very valuable, containing a single area with almost a third of the total number of trees mentioned on this list, is represented by the area owned by the National Bank of Romania, located near the pier of Dambovita, better known by citizens as the place of sports competitions NBR Arenas. The 31 trees of the Platanus x acerifolia species present in this site require thorough research, are considered, in an advanced state of decay. Our study aims to raise awareness and advertise, this concentrated protected historic landscape site, in order to initiate a complex process of integrated and differentiated management of the tree vegetation of cultural identity and social values.

Key words*: regeneration, rebirth of value, urban historical reintegration, trees protection.*

INTRODUCTION

Urban green spaces have a large contribution in defining the quality parameters of social and cultural life, being a dynamic component of environment, under constant urban evolution at the same time with the society evolution.

Generally, we can talk about two basic senses of the quality notion:

-quality based on the understanding of the Aristotelian sense of the “quality-species” term, which defines the sense of being of a thing, as nature, the essence of the thing;

-quality defined by considering a thing like “good” or “bad” defining the “quality-value” term.

At the same time, we can talk about the primary and secondary qualities of landscaping, defined in terms of philosophy, the primary ones being intrinsic qualities, of real things and the secondary ones being those that have the power to bring us certain feelings (Stănescu, 2008). All these relations determine another category of quality, more special for a city, namely its inner energy, which defines and characterizes a city (Sandu, 1992).

In a city, urban energy and quality of life is supported and determined in a large scale by the dynamic component of the vegetation too, especially by tree vegetation, playing its part in regulating the urban ecosystem. The world becomes richer due to the memorable places where people design their life and constructions (Simonds, 1967). The ecological and cultural-historical principles of the building and analyzing urban landscapes lead to conservation and preservation measures, in case of valuable urban landscaping.

The management, based on the analysis and control of urban landscaping development, allows the controlled evolution of vegetal assemblies, based on the principles of globality and continuity of the analysis, assessment and intervention over the vegetation.

Provided that the urban space has special features, given by the historical, memorial, ecological or social value, the urban vegetal assembly becomes heritage which can help in the interpretation of the society evolution, by understanding social, aesthetic and cultural concerns.

In the heart of Bucharest, very close to the “heart of the city”, on the right side of Dâmbovița River, we have a very valuable landscape, dominated by some special historical plane trees, of the *Platanus* × *acerifolia* species*,* which actually gave the name of the land - the Plane Tree Park. The piece of land suffered progressive changes, currently belonging to the National Bank of Romania.

The current study aims to investigate the evolution of the site, analysis of the history and continuity of the site, identification of trees with historical, ecologic, social and memorial value, control and update of information included in the List of protected trees, related to the *Platanus* × *acerifolia* trees existing in the Plane Tree Park. After the heritage trees have been identified, the study aims for the future to continue the researches, to achieve a complex analysis of the dendrometric characteristics of each tree, determining the functional, aesthetic and memorial value in order to preserve and protect the heritage of this site.

All researches and analysis on the field and in the archives will lead to specific measures for the preservation and regeneration of the historical landscaping in the Plane Tree Park.

MATERIALS AND METHODS

Urban landscaping can be considered a symbol, a sensible unit, which interpretation is determined by the cultural experience, respectively of practices and events specific to communities (Majuru, 2012). At the same time, it is a specific layer accumulation of several practices, policies, customs, events which builds the memory of the place (Tudora, 2009).

Amid cultural accumulation and territorial changes, the site investigation method was focused on two important issues:

- studies and historic analysis of the landscape structures of the site - analysis of the urban area;
- studies dendrometric and visual analysis of the secular plane trees on the studied site.

The historical analysis was based on the archival studies of the place history, completed with the study and analysis on the field of the existing structure. The historical study analyses the evolution of the current territory of the Plane Tree Park, in order to identify the specific moments during its existence, as well as in order to raise awareness on the landscape heritage value of Bucharest.

The historical plans of Bucharest have been analyzed (1846, 1852, 1871, 1895, 1899 and 1911), together with the literature in terms of evolution of the public urban green space and of gardens of Bucharest.

Historical research started with the analysis of the most important structures - roads, benchmarks, nodes, limits, specific typology areas - which defined the mental map (Lynch, 1960), but also the physical and evolutional map of the place.

The historical analysis also indicated how the secular vegetation of the Plane Tree Park started and developed in terms of urban and social conditions on this location.

Nevertheless, the complex landscaping analysis methods conducted on the field referring to the historical vegetation were focused on dendrometric measurements, analysis to identify the aesthetic, historical and landscape values, on considering the state of health of the analysed trees.

A precise survey was conducted with the position of all plane trees, each tree analyzed was assigned a unique registration code, according to the vegetation inventory method (basic tool in Management of Urban Vegetation).

Tomography analyses were conducted for the internal structure of each historical plane tree log existing in the analyzed site. This complex analysis helped in identification of the internal gaps, of wood firmness, and finding hidden cavities which affect trees’ stability and health.

All assessments and analysis performed were scientifically construed and they were considered as the first database organized on scientific grounds of the Plane Tree Park. This particularly important document is the main tool used to prepare the Management and Integrated Plan of the Plane Tree Park.

RESULTS AND DISCUSSIONS

Analysis and historical studies

The analysis and interpretation of the historical plans indicated the evolution of the analysed space, from the peri-urban area to the modern area, emblematic of today's society.

In terms of historical period evolution, we can see the stratification of at least six determined period, with insignificant overlaying of years, when the current territory undergone significant changes:

- the first period -until 1870, indicates us a peri-urban area, affected by the constant flooding of Dâmbovița. The area is left somehow not systematized because the city not included Dâmbovița River. Starting with 1800, Bucharest inhabitants begin to capitalize the river's adjacent spaces, for leisure purposes. The land under study was overlapping the semi-agricultural piece of land existing almost two centuries ago in this location (Figure 1).

Figure 1. Bucharest Plan in 1846 - taken over from the Borroczyn Plan - according to the redo of drawings in 1911 coordinated by Cincinat Sfințescu (Source: www.ideiurbane.ro)

-the second period - from 1962 until 1895, when the first systematization of Dâmbovița river occurs and the land is designed as target range. The project coordinated by Major Papassoglu shows a space organized with an alley, bordered by a bilateral tree alignment, leading to the shooting gallery - "Tiru Gherman" (Figure 2). Dâmbovița is regularized, drained, registered in the land of the "Societatea de Dare la Semn", established by the ruler Cuza in 1862, the access was done by Notagiilor Street, part of the current Dr. Staicovici Street.

Figure 2. Bucharest Plan in 1871 - taken over from the Papassoglu Plan - detail of Green Color (Source: www.ideiurbane.ro)

-the third period - since 1890 until 1911, when final rectification of Dâmbovița takes place and when the main boulevards are drawn in the urban structure. We can see that the land of the "Societatea de Dare la Semn" is organized with alleys, pavilions, plantations and a pond. The streets and lots of the new district, Cotroceni are drawn (Figure 3 and Figure 4).

-the fourth period – 1912, the area is partially rethought in terms of functionality, the park is shared by Tenis Clubul Român (Romanian Tennis Club) and Societatea de Tir (Shooting Gallery Company), the previous landscape is changed.

-the fifth period - the interwar period - it does not bring any radical changes, the neighborhood is defined as street scanning field, Dr. Lister Streets appear, when the main entrance of Romanian Tennis Club is moved. In 1939, the tennis courts were taken over by ANEF, whipped out by the new political regime, nine years later.

Instead of the pond, we can see now a modern pool, on the east side, which will be opened until 1962.

-the sixth period from 1945 until 1990 – define the current configuration of the land, the Cotroceni Stadium is built (1950), the new

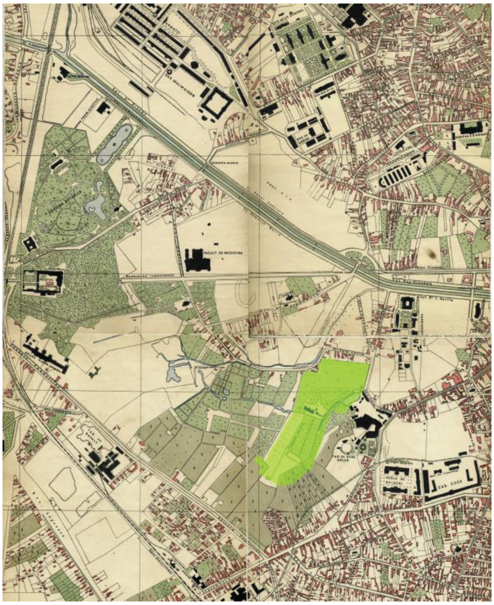


Figure 3. Bucharest Plan in 1895 – 1899
(Source: www.ideiurbane.ro)

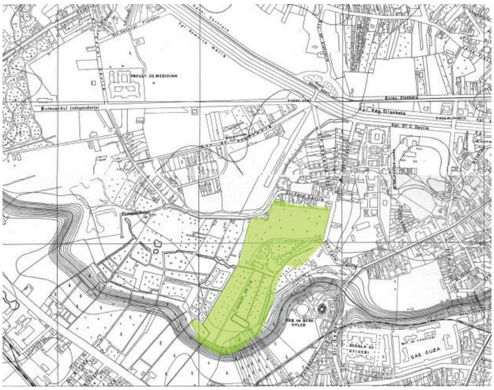


Figure 4. Bucharest Plan in 1911
(Source: www.ideiurbane.ro)

center of Victoria Socialismului is drawn and built now (1980).

Currently the piece of land can be accessed on the two main entrances (from Dr. Lister Street and Dr. Staicovici Street), a complex scanning field of roadway and pedestrian walkways, which serves the tennis courts, football fields, office spaces, sports halls, restaurant, guest house, outbuildings and annexes, seating areas, pavilion, gazebo, leisure areas.

Vegetation analysis

Valuable tree vegetation, of historic trees - *Platanus × acerifolia,* the species that named the place - the Plane Tree Park - it has 31 monumental trees, of different health stages.

The List of Protected Trees, under the administration of Romanian Academy, holds 39 historic trees of *Platanus × acerifolia* out of the total of 110 trees listed and inventoried in Bucharest. Out of the total of 39 protected plane trees, 31 are to be found in the Plane Tree Park, counting for almost 80% (79.8%). At the same time, out of the total of protected trees in Sector 5, more than 85% are located in the Plane Tree Park (Figure 5 and Figure 6).

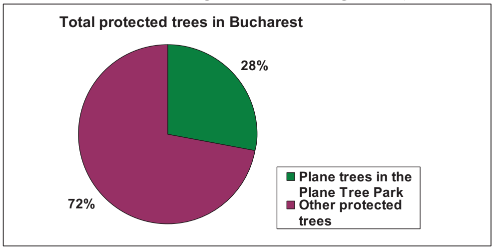


Figure 5. Percent of plane trees out of the total of protected trees in Bucharest

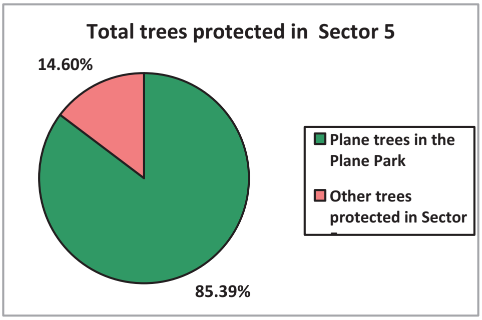


Figure 6. Percent of plane trees out of the total of protected trees in Sector 5 Bucharest

The analysis started in 2014 generated a Specialized Technical Expertise Report which indicated the health condition of the protected plane trees. Three major categories were identified for the physical health condition of the trees identified in the topographic plan: good, average and poor (Figure 7).

Each tree was analyzed individually following a scheme drawn according to the principles of Sustainable Management, issuing an Individual Analysis Sheet.

The following elements were analyzed individually: tree morphology (basic anatomical elements: stem, bark, crown), internal structure of the stem (considered following the tomography analysis conducted), the health condition and aesthetic appearance.

Figure 7. Identification plan of the protected trees, assessment of their physical health condition

Technical Expertise Sheet for woody plants

Aria code: **11934**
Zone code: **A**
Tree code: **11934-A-Pa02**
Tree code on map: **A-Pa02**
Specie: **Platanus x acerifolia**
Protected Tree: **yes**

1. Dendrometrical measurements

Tree code	Circumference (m)	Height to crown base (m)	Crown width (m)	Total tree height (m)	Trunk numbers	Number of limbs
11934-A-Pa02	3.3	2.5	14	15	3	6

Aesthetic appreciation

Tree code	Aesthetic value	General tree condition	Stability	Recommended pruning
11934-A-Pa02	medium	good	yes	rejuvenation

Figure 8. Individual Specialized Technical Expertise Sheet with dendrometric recording and aesthetic analysis

Each *Individual Analysis Sheet* includes a series of recommendation and interpretations of the results analyzed and the measurements conducted (Figure 8 and Figure 9).

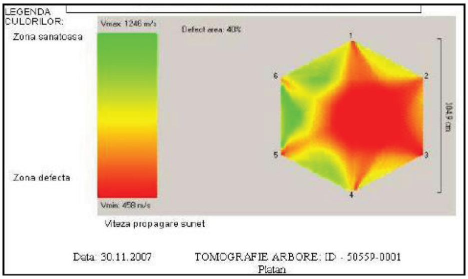


Figure 9. Individual Tomography Expertise Sheet

The historical trees were analyzed in full, all existing diseases and affections, growing and development anomalies were identified, as well as the condition and balance of the morphological elements. All information was recorded in hard support format and photographic documents, in order to set a customized diagnosis, for a tree to be given the proper treatment to improve its health condition (Figure 10).

Figure 10. Analysis, diagnosis and individual treatment sheet

A part of the trees analyzed were seen in an advanced state of decay, as the main parts supporting it were not present. It meant they had to be removed, being considered as imminent hazard of safety for the buildings and pedestrians (Figure 11).

Protected trees, with significant health problems, in an advanced physiological state of decay (but with great historical value) were mentioned in special sheets (but not recorded as vegetal elements) under provisional preservation, with mandatory constant monitoring and they can be cut at first sign of possible hazard. The individual analysis sheet indicates actions to be taken to increase their safety and stability (Figure 12).

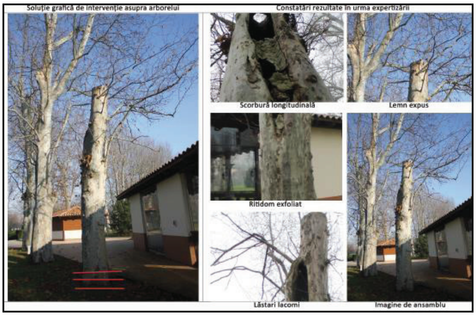


Figure 11. Hazardous Trees Identification Sheet

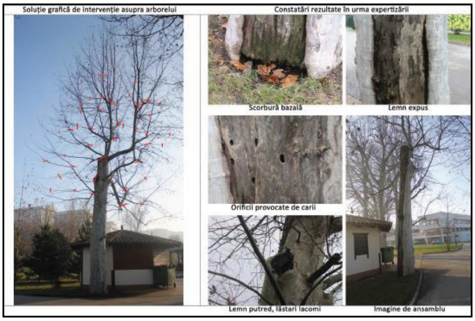


Figure 12. Individual sheet of protected trees with special monitoring condition

Out of the total of 48 plane trees analyzed, only 31 trees are noted in the List of Protected Trees. The individual analysis of the trees present in the studied site indicated that there are trees with the same landscape and historical value, but which are not mentioned in this list (Figure 13).

Figure 13. Individual analysis of the trees with similar value as the protected trees

Careful analysis of each tree provided primary information, centralized in complex inventory sheet. They include data related to the surface code, analyzed tree code (unique and individual), diameter of the trunk (measured at 1.3 m from base), crown inserting height, diameter of the crown, total height of the tree, number of branches (main, secondary), aesthetic value, intervention required, general health condition (pruning, treatment), type of intervention recommended, root system, approximate age (Figure 14 and Figure 15).

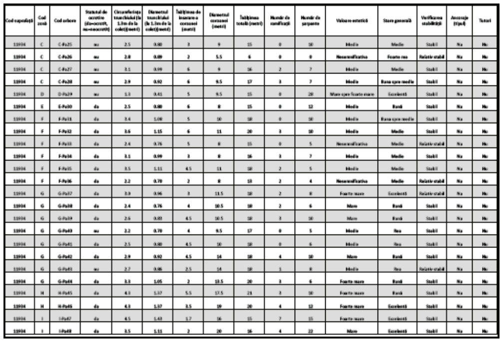


Figure 14. Inventory Sheet

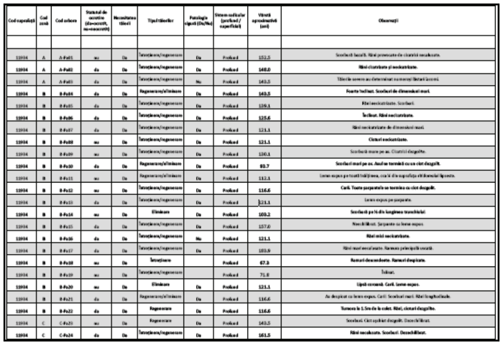


Figure 15. Inventory Sheet

The results of the research indicated valuable vegetable elements that are not protected, but also the presence of protected vegetal elements whose value is diminished, in low, irreversible psychological condition.

CONCLUSIONS

The impressive number of historical trees grouped in the land assessed can give us an idea about the importance and the heritage value of the analyzed site.

The valuable tree vegetation in the Plane Tree Park counts for almost 1/3 of the total of trees listed in the Protected Tree List (31 trees, out of the total of 110).

Nevertheless, another 17 trees of *Platanus* × *acerifolia* species found and analyzed in the site, have aesthetic values and dendrometric consideration as the protected ones (Figure 16).

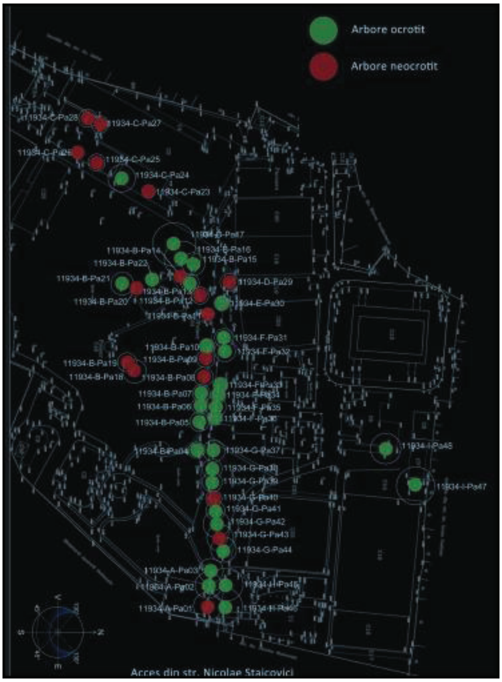


Figure 16. Plan for the identification of the protected trees

In total, the number of trees that can be considered protected, could be of 48, not 31, as they are currently listed in the Protected Trees List.

The classification of the trees into the Protected Trees List could follow a similar procedure listed in the methodological norms for the classification and inventory of historical monuments. This procedure is based on classification criteria, which assess the cultural significance and importance of the immovable assets, establishing the legal category and valuable group of the national cultural heritage which these assets belong to, respectively group A or B of historical monuments (landscape). Therefore, the classification of an immovable asset is conducted based on the following criteria:

a. Seniority criterion.
b. Criterion related to the artistic, urban (landscape) value.
c. Criterion related to the memorial and symbolist value.

These criteria, applied in line with the legislation of architectural historical moments could be slightly adjusted in order to be applied for landscaping of historical monuments.

The general health condition of the trees analyzed, protected or not protected, is poor, most of them indicating early signs of aging. A part of the trees existing, mainly historical plane trees, was presented even in the first stage of the site analysis (starting with the 19^{th} century). The plane tree is a long-lasting species and the estimated age of the oldest trees (max. 180-200 years), does not explain the advanced grade of their physiological decay.

Related to the phenotype description of the species, significant differences were registered between the typo species and the species analyzed. Most of the trees present of the land indicate mainly vertical growing, while the typo species has large crowns, with horizontal growing.

The trees stability analysis indicated as main issue the internal cavities or even open cavities, some of them noticed on the field, others indicated following the tomography analysis.

Previous interventions (infilling, cutting) were severe, the trees reacted in uncontrolled growing of greedy springs, concurrent branches, terminal ends, with the internal wood exposed. All these interventions lead to the worsening of the plane trees physiological health condition.

All the trees examined were diagnosed and recommendations were issued for regeneration pruning and balancing of crowns, of disinfection and infilling of hollows and open cavities.

The analysis and measures were taken and organized in the first Tree Inventory Register, a basic document in planning the interventions and Landscaping Sustainable management. The integrated landscape management survey indicated the importance of constant monitoring of valuable plane trees, but also the program of required interventions, as a matter of emergency or immediate operations. The scientific system for the registration and inventory of each tree as settled. The results of the survey indicated 8 trees of *Platanus* × *acerifolia* species in a very poor condition, some of them should be cut immediately as there are facing a constant hazard of falling. In the following current management stage, we will update the analysis of vegetation - once in 5 years - and the tomography analysis will be redone for the constant monitoring of the health condition of the historical plane tree in the Plane Tree Park.

ACKNOWLEDGEMENTS

This survey was conducted with the support of the National Bank of Romania, through the project "Integrated Management and Administration of the Plane Tree Park -owner NBR."

REFERENCES

Lynch K., 1960. The image of the city, The MIT Press, page 20-60.

Sandu Al., 1992. Structura urbană, Curs, cap. III, Ed. UAUIM, București, 18.

Simonds J.O., 1967. Arhitectura peisajului, Ed. Tehnică, 53.

Stănescu A., 2008. Aspecte ecologice ale calității spațiilor verzi - implicații în peisagistica urbană, Ed. AmandA Edit, București, 16.

Tudora I., 2009. Teoria peisajului. Definirea interdisciplinară a peisajului. USAMV, 112.

***www.ideiurbane.ro

***The methodological norm for classification and inventory of historical monuments dated April 18, 2008.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INITIAL PLANTING DESIGN OF THE CAROL I PARK IN BUCHAREST

Ileana Maria PANŢU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: ileana.pantu@gmail.com

Abstract

This paper aims to analyze the planting composition of the Carol I park in Bucharest at the beginning of the 20th century. The Carol I park is important for the history of Romanian landscape architecture and stands also as an example for the design of parks nowadays. Conceived in 1906 as a national park to host an international event, the "General Romanian Exhibition", The Carol I park was transformed throughout the 20th century to represent symbolically different political powers. A royal showcase at first, it then became a tool of communist propaganda in the '60s. The park's compositional style also changed with its ideological mutations and its planting design followed. It is essential to analyze and understand the evolution of parks and especially the evolution of their planting design in order to valorize and preserve and/or restore national landscape heritage. The first stage of the history of the Carol I park demonstrated an elegant Belle Époque style with both Romantic and geometric areas designed by the French landscape architect Édouard Redont, specially invited for the abilities he had already demonstrated in Romania. The composition of the park was centred on a generous circulation space with lots of pavilions, interspersed with water elements. Elegantly planted in a geometric style with a lot of attention to detail, this axis has continued visually towards a hill over a sinuous lake. On this hill, the eye is drawn to the Palace of the Arts. Below it, an elegant coniferous composition engulfed a graceful Romantic grotto with a high cascade guarded by a sculptural ensemble. The alleys and the vegetation near the lake and on the hill were designed in the French landscape style, in contrast with the geometry of the axis zone and its side alleys, with alignments of trees, pruned shrubs and flower platbands.

Key words: *Belle Époque style, geometric style, planting design, public park, Romantic style.*

INTRODUCTION

This paper will analyse the planting design of the first stage of the evolution of the oldest and one of the most important public parks in Bucharest, the park Carol I. Conceived in 1906 as a national park to host an international event, the "General Romanian Exhibition", a tribute to Carol I, who had then reigned for 40 years (Parusi, 2007; Potra, 1990; Panţu, 2011), the park was transformed in the '30s (Panţu, 2011) and more radically in the '60s in order to eradicate its royal symbols and become a tool of communist propaganda (Panţu, 2015). Thus, the park changed in compositional style, including its planting design, over its ideological mutations. The history and morphological transformations of the Carol I park demonstrate all of the Romanian landscape styles of the first half of 20th century in just one park (Panţu, 2012).

The French landscape architect Édouard Redont was invited to design the park due to the great abilities he had already demonstrated in Romania (Teodorescu, 2007). He created an elegant *Belle Époque* park in a mixed style with predominant Romantic style - French landscape style, and a geometric part from the entrance zone to the heart of the park (Panţu, 2011) (Figure 1).

RESULTS AND DISCUSSIONS

Redont conceived a mixed style planting design, with a large part in the Romantic style, where he grouped the plants so as to resemble natural landscapes (Figure 1).

The composition of the park was centred on a generous circulation space with lots of pavilions – a large parterre with two lateral alleys shaded on both sides by alignments of linden trees (Figure 1). Redont treated in an architectural, geometric manner, in a classical style, the plantation along this main axis: the symmetry of the parterre with bowling green rhythmed with spherical box and yew, borders of ornamental leaf plants, flower platbands, tree alignments etc. He designed the rest of the park in a Picturesque, Romantic style: trees and shrubs in free shaped masses, in groups or isolated, composed in order to create varied sequences, profusions, clever effects of light and shade etc. The main axis continued visually over a sinuous lake towards a hill with the imposing building Palace of the Arts that dominated the entire park. Below it, a grotto with cascade and a sculptural ensemble was the heart of the Romantic area (Figure 1).

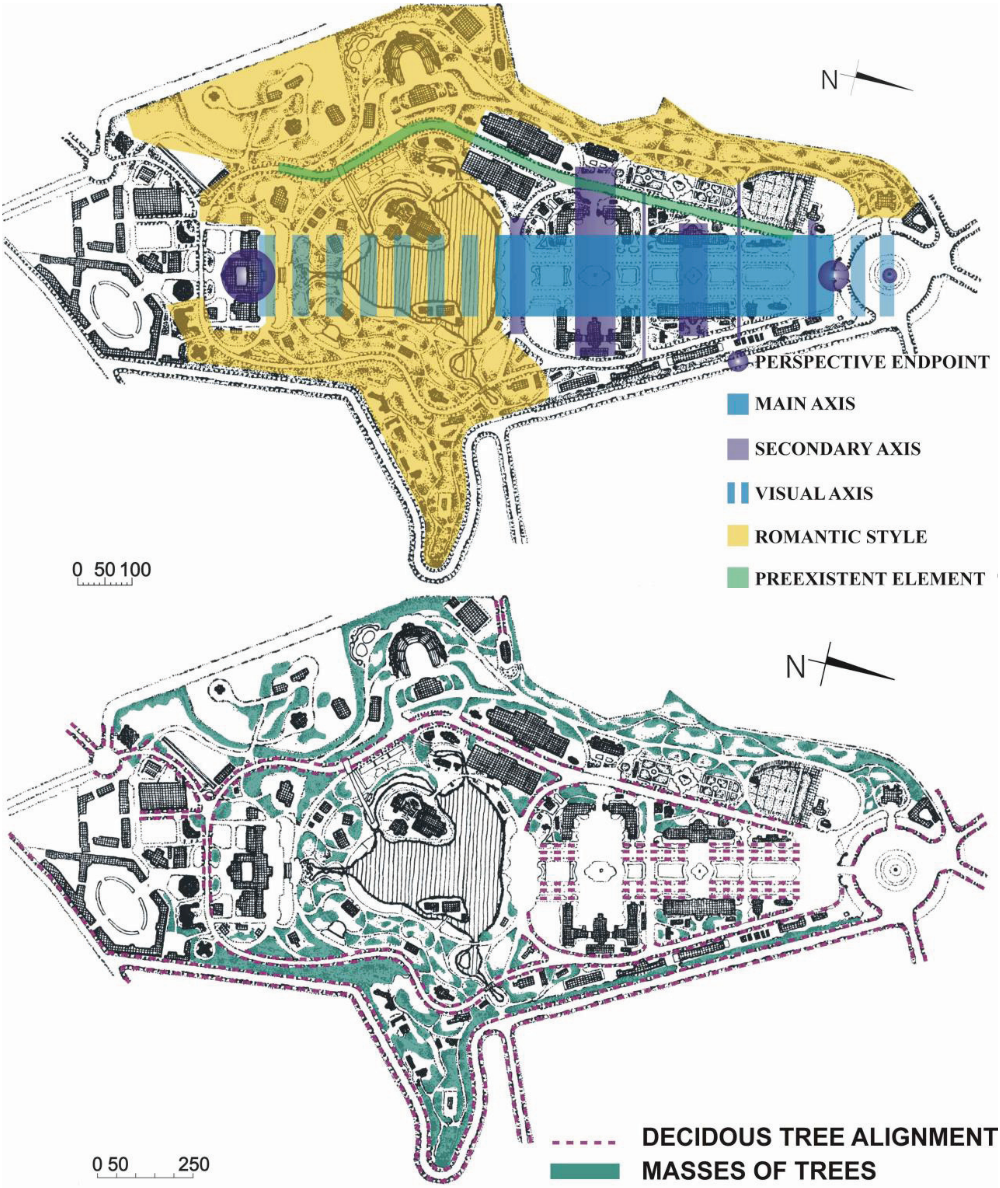


Figure 1. Composition and vegetation structure of the park Carol I in 1906 (Panţu, 2011; Marcus, 1958)

In order to create the planting design, Redont diligently searched Bucharest and discovered valuable mature tree species. Their vitality, their habit, the colour of their foliage, their blossom or fruit offered total success to one who knew how to find the right place to set them off. Redont's inventory stands as evidence for that (Teodorescu, 2007). In addition to the plants brought from nurseries, these species numbered about 6,000 resinous trees, 4,200 high, mature deciduous trees, 90,000 young trees (Teodorescu, 2007; Bulei, 1990), 48,200 shrubs, 49,200 forest plants, 8,400 plants with varied flowers, 98,000 flourished plants and 3,500 kg of grass seed (Potra, 1990; Panţu, 2011). All of these species conferred to park visitors all the benefits of a vegetation at least partially mature, such as shade so welcome in the hot Bucharest summers.

In the general framework, coniferous outnumbered by far deciduous trees (1.43 times), unusual for a park situated in the plain (Figure 1). Nevertheless, the large difference in level in the park site (Filaret Hill) amplified by Redont, by modelling the landscape and the usage of rockeries and abrupt vegetation created a mountain-like image and so allowed the resinous trees to dominate the view (Panţu, 2011, 2012).

Redont retained in the park's composition the former road that traversed the site as an important side alley with its double alignment of *Populus alba* - the oldest trees in the park, giving a lot of shade, necessary in the Bucharest summers (Figure 2). These white poplar lines are easily distinguished in all the early aerial views of the park. (Figures 3-5).

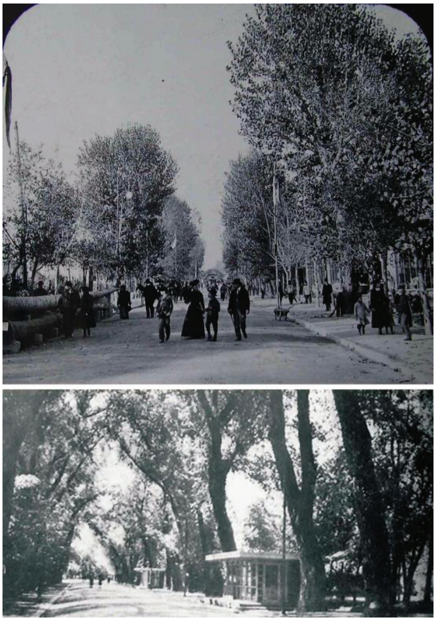


Figure 2. Former Filaret Avenue in 1906 (Zaharia, 1906) and late '50s (Marcus, 1958)

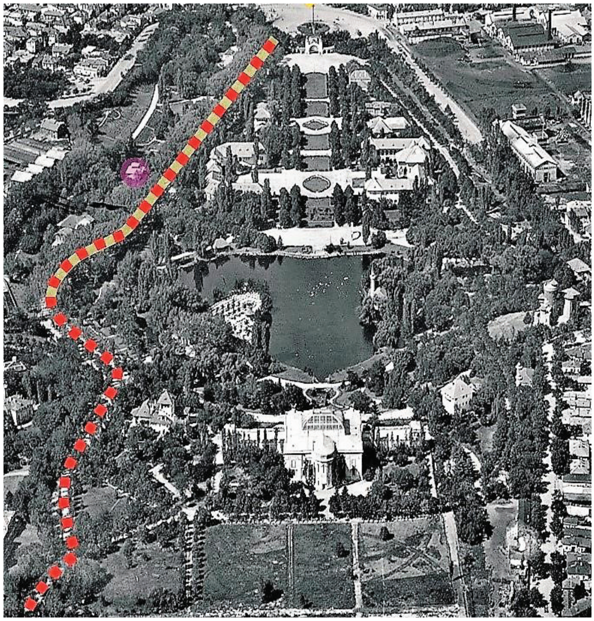


Figure 3. Visual analysis of the park Carol I in 1927: red - alley on the former Filaret Avenue with its white poplars alignment - yellow (Panţu, 2012; Drăgan, 2006)

The roads edging the parterre in the main axis were flanked on both sides by alignments of linden trees – young at plantation time (Figures 1, 8). On the outside margins of the axis, Redont put a line of more widely spaced columnar poplars alongside the linden trees, creating interesting vertical accents (Figures 4, 5, 7, 8). He used these species also in other areas of the park, especially on the lake shore, conceiving great compositional effects (Figures 14, 19).

On the eastern side of the park Redont designed an alley symmetrical to the former Filaret Avenue. He shaded it with a quadruple alignment of chestnuts a few of which remain today (Figure 4).

The axis was underlined by the horizontality of the linden alignments foliage, of the columnar poplars curtain and of the old white poplars in the background, as well as by the linearity of the parterre and of the box borders and box spheres that rhythmed its long edges (Figures 4, 5, 7, 14).

Redont placed spherical box not only along the axial parterre sides, where it highlighted the classical style, but also along secondary alleys to smooth the transition to the Romantic areas of the park (Figure 6). Some of these spheres still remain nowadays thanks to the well-known longevity of box (Figure 6).

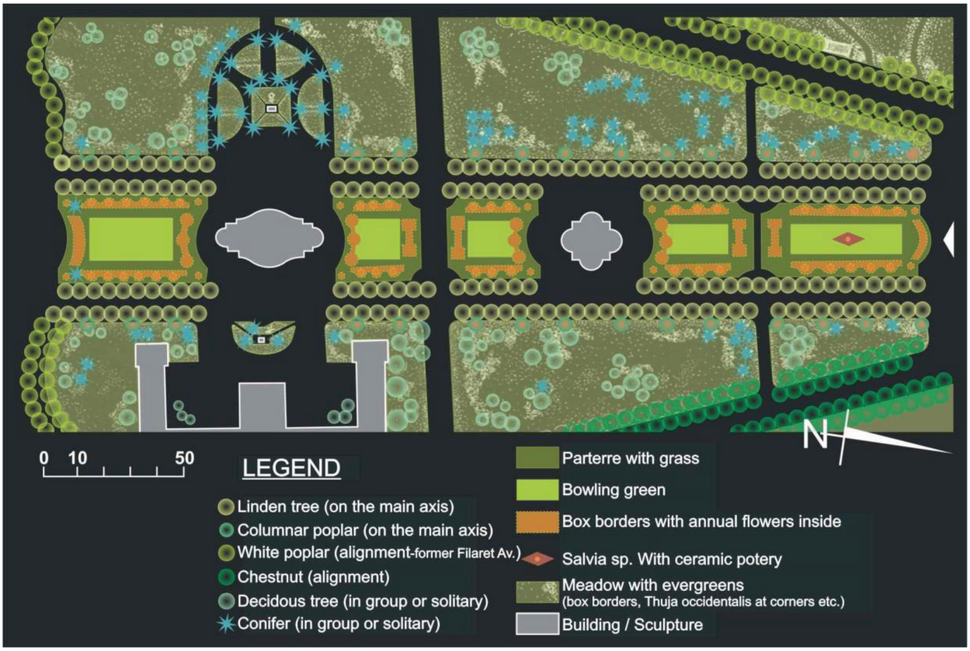


Figure 4. The main axis plan (Panţu, 2012; Marcus, 1958)

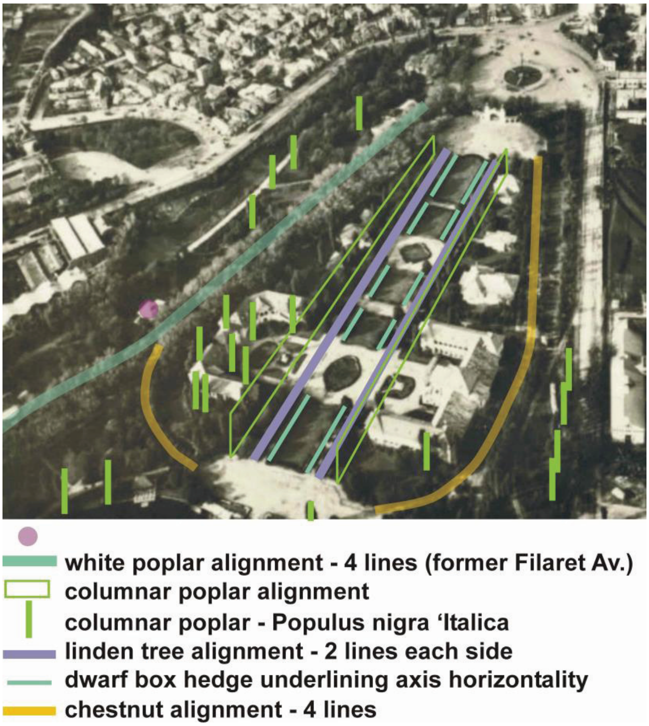


Figure 5. Analysis of 1930 vegetation (Panţu, 2012)

Figure 6. Box spheres along curved alleys in 2011

Figure 7. The main axis with the pruned linden trees, 1938

We believe that the quadruple linden alignment on the main axis was geometrically pruned in 1935, on the occasion of another important exhibition that brought also other transformations to the park analysed in the paper *Carol I Park in Bucharest in the '30s – Celebrate Bucharest Month* (Panţu, 2011). The crowns formed long prisms, typical of the classical French style (Figure 7). Our arguments are that the intention of geometrization of the linden trees does not appear in the images until 1935 (Figures 3, 5), and becomes evident in the photographs from 1935 onwards (Figure 7). It is possible that the pruning of the alignments was part of Redont's vision for the future, as it was not possible in 1906 when the lindens where far from mature. The intention of pruning was still visible in the late '50s, but subsequently lost.

The vegetation details in Figure 8 from 1906 are in the same range of classic elements. The linden trees are young and the parterre with geometrical bowling green is contoured with small hedges in light coloured foliage plants and flowers. Those borders are punctuated with sphere shaped yew or box. The parterre corners were accentuated with *Canna indica*. Later, in the'50s, the ornamental details of the central parterre retained the same French classical spirit (Figure 9): strips marking its contour in *Begonia* and double borders in dwarf box, with accents of *Canna indica* and *Yucca filamentosa* etc.

Figure 8. The axial parterre and the Industries Pavilion at left to the entrance (Zaharia, 1906)

Figure 9. Planting design in a corner of the axial parterre in the '50s (Marcus, 1958)

Figure 10. Decorative basin on the main axis in 1928

In 1928 the neoclassical outline of the decorative basins was valorised by floral bands of *Canna indica* and *Tagetes* (Figure 10). The border of the basin in front of the Royal Pavilion was also accompanied by *Canna indica* (Figure 11).

Figure 11. Decorative basin on the main axis in front of the Royal Pavilion

We believe that there was a predilection for *Canna indica,* that can also be identified in other designs of the time, especially around basins, not only in Romania, but also in France, where, in our view, this fashion emanated at the end of 19th century. For example, The Buffet of the Avenue in Bucharest (a heritage building from 1892 by arh. Ion Mincu) (Figure 12) and a fountain in an important plaza in Bordeaux (Figure 13).

Figure 12. The Buffet of the Avenue in Bucharest

Figure 13. Fountain on Allées de Tourny, Bordeaux

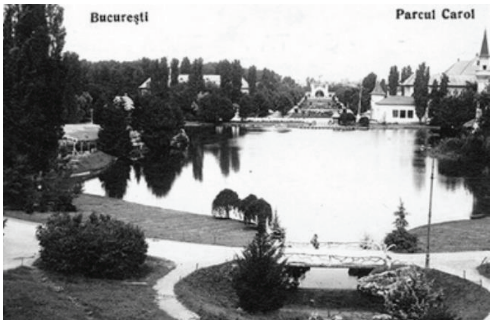


Figure 14. The main axis from the Palace of the Arts (Romanian National Library Archive)

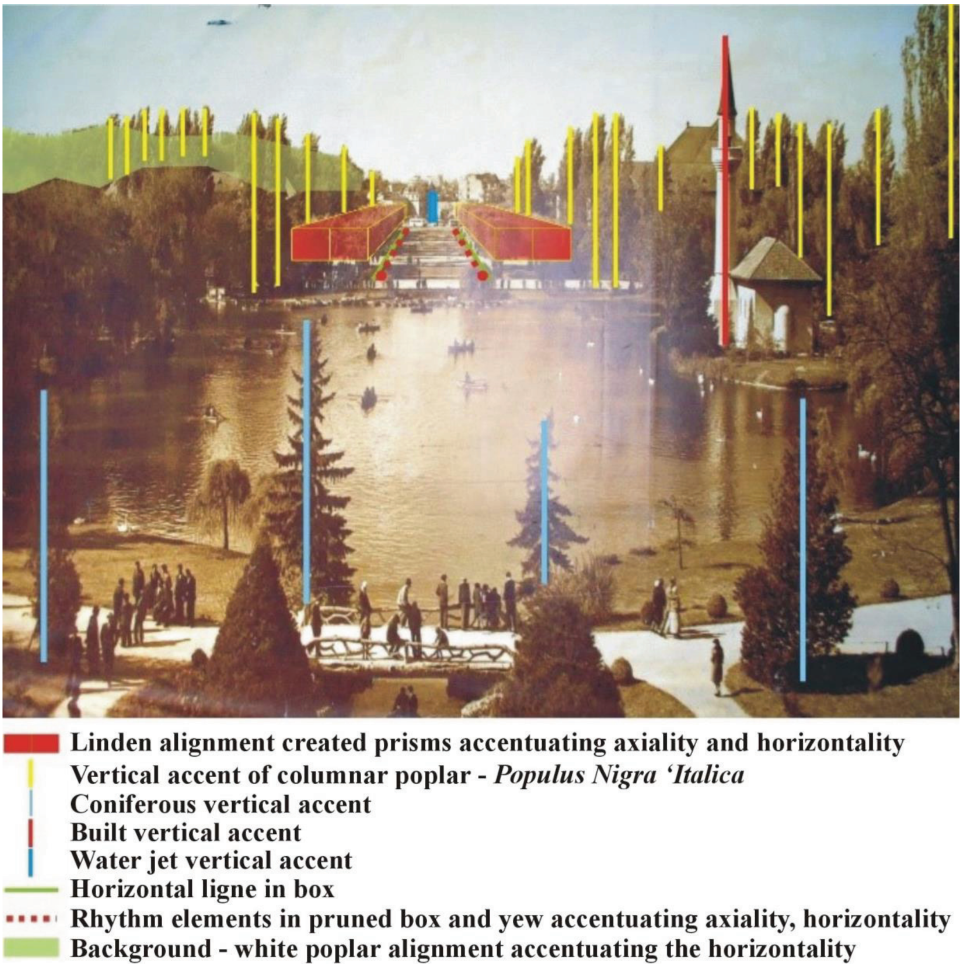


Figure 15. Visual analysis in 1935 (Panţu, 2012)

The most visible vertical accent in the Carol I park in the '30s was the minaret clearly distinguished not only on the sky by the contrast with the dark colour of its top, but also against the background vegetation by the difference in texture and colour and on the lake. Its verticality was underlined by the lake's horizontality and by dark foliage and a slender conifer that Redont placed in front (Figure 15). So, the minaret constituted an interesting landmark visually, but also culturally, as a Romantic park folly.

Vertical accents were placed also on the other side of the lake – unaligned, as a Romantic figure. Two spruces asymmetrically disposed framed the perspective of the axis towards the main entrance. The composition was balanced to the visual axis and so were all the vegetal elements (Figure 15).

The main axis continued visually over the sinuous lake towards a hill – the Romantic area. Here a balanced composition took the place of symmetry. Each element had something to balance it on the other side of the visual axis: the two slender spruces on the lake shore, the weeping trees on their side, the voluminous conifers with compact foliage – pruned yews or compact horticultural forms – the bold cypresses, the small shrubs at the ends of the Romantic bridge imitating tree branches (Eiffel pattern) etc. (Figures 14, 16). The group of three weeping trees - *Ulmus glabra* '*Pendula* - formed an interesting presence on the lake shore (Figures 14, 16). Redont placed this variety of elm elsewhere in the park also, for its spectacular shape.

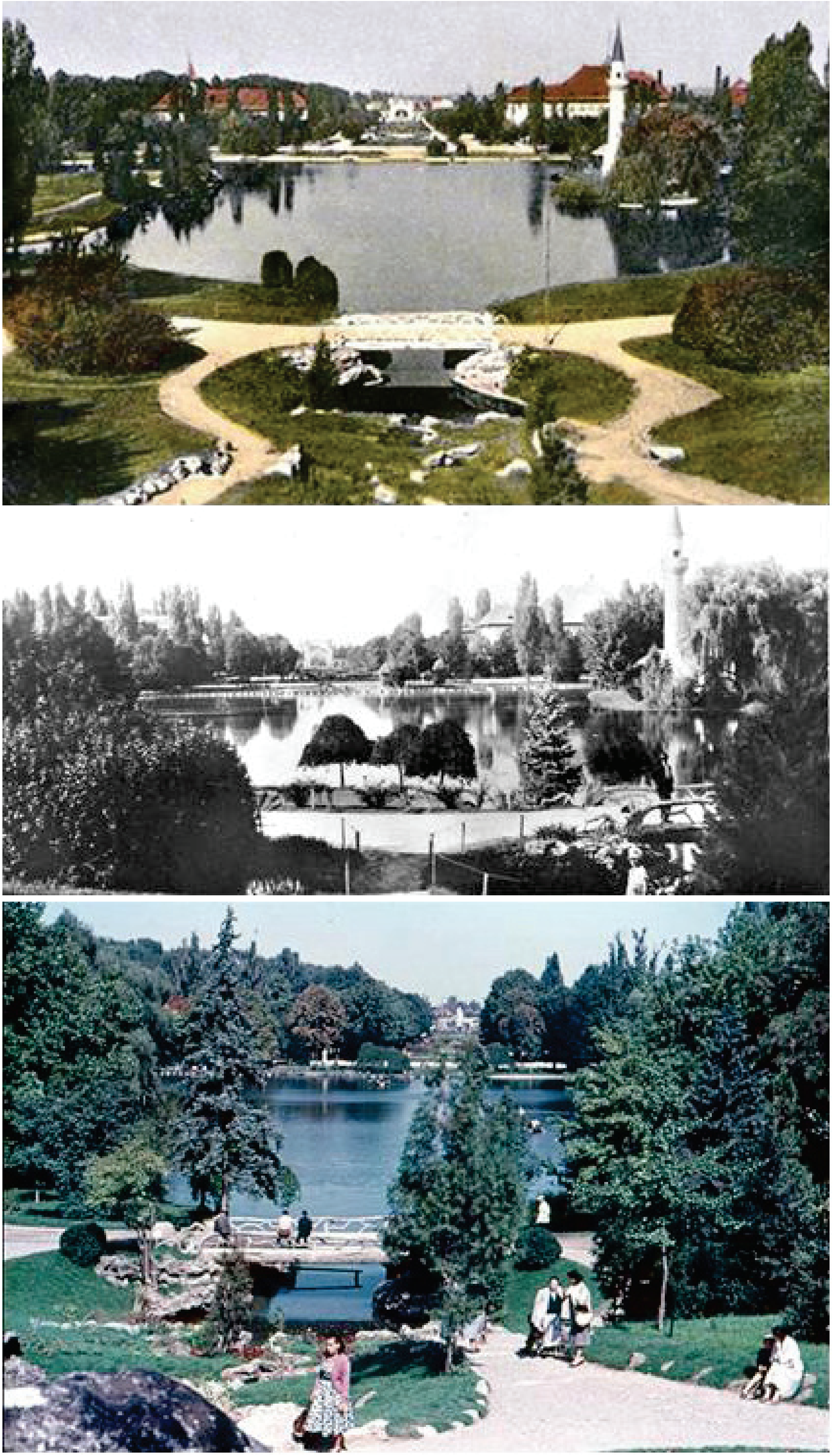


Figure 16. Views from the grotto towards the main entrance around 1910, in 1928 and in the ‘50s (Panţu, 2012; Octavian and Georgescu, 1999; A.F. Iliescu archive)

Beneath the Palace of Arts, around the grotto, Redont conceived a vegetal composition in conifers only, appropriate to the rockery articulating different height levels and so creating a mountain-like image on the steep slope. Dense habit, voluminous conifers were balanced disposed to the main axis (Figure 17).

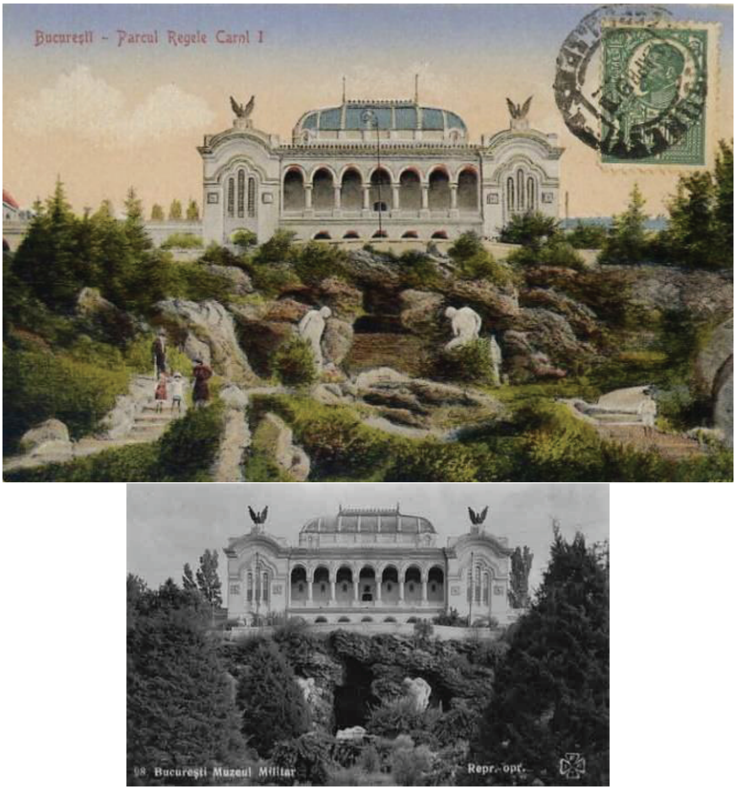


Figure 17. Coniferous composition on the hill beneath the Palace of the Arts around 1910 and later, in 1937

Redont designed the plantation in Romantic style also in the surrounding park areas, on the lake shore and on the slopes. He composed it in elegant tree and/or shrub masses and groups and solitary specimens with which he created savant landscape sequences, in a clever scenography (Figures 17-19).

The lake shore was decorated with black and columnar poplars, willows, bold cypress spectacularly colouring the park landscape in autumn (Figures 16, 18, 19).

Figure 18. The lake, the mosque and the palace

Figure 19. The eastern lake shore in the ‘50s

Under the communist regime, in 1960, the Carol I park underwent radical transformations and lost a great part of its Romantic style in favour of a monumental geometric style. As a consequence, a lot of the plantation from 1906 disappeared. The percentage of original plantation from 1906 that has lasted until nowadays is small, about only 10%. I believe that this was caused also by the passing of time over 100 years, lack of vegetal protection strategy, bad heritage park management and faulty maintenance.

CONCLUSIONS

The percentage of the 1906 plantation that had lasted until nowadays is small, about only 10%. I believe that this was caused by the radical transformations in 1960, passing of time over 100 years, lack of vegetal protection strategy, bad historical park management and faulty maintenance.

Planting design is most important for overall composition and shows the characteristics of respective styles. It is primordial to understand the initial design of the Carol I park in order to comprehend, then preserve and/or restore the national landscape heritage.

REFERENCES

Bulei I., 1990. Atunci cand veacul se nastea… Eminescu Publishing House, Bucharest.

Drăgan V.E., 2006. Studiu Istoric pentru Parcul Carol I. Analiza valorilor culturale ale imobilului şi ale zonei învecinate. Reguli de intervenţie pentru conservarea valorilor culturale şi integrarea în zonă.

Marcus R., 1958. Parcuri şi grădini din România. Tehnica Publishing House, Bucharest.

Octavian T., Georgescu M.P., 1999. Inter-Bellum Bucharest, CD, NOI Media-Print, Bucharest & D.O.R. Kunsthandel, Olsberg.

Panţu I.M., 2011. Carol I Park in Bucharest at the Beginning of the 20th Century. in USAMV Bulletin Horticulture, 68 (I)/2011, Cluj-Napoca, 400-407.

Panţu I.M., 2011. Carol I Park in Bucharest in the ’30s – Celebrate Bucharest Month. Scientific Papers. Series B. Horticulture – vol. LV 2011, Bucharest, 310-315.

Panţu I.M., 2012. Parcul public bucureştean din secolul XX. Influenţa modelului francez. Doctoral thesis

Panţu I.M., 2015. Carol I Park in Bucharest in the Second Half of the 20th Century. Scientific Papers. Series B. Horticulture – vol. LIX 2015, Bucharest, 385-392.

Parusi, Gh., 2007. Cronologia Bucureştilor. Compania Publishing House, Bucharest.

Potra, G., 1990. Din Bucureştii de ieri. Ştiinţifica şi Enciclopedica Publishing House, Bucharest.

Teodorescu V. Z., 2007. Un parc centenar. Parcul Carol I. Muzeul Municipiului Bucuresti Publishung House, Bucharest

Zaharia A., 1906. Exposiţiunea Generală Română din 1906 fotografiată de Al. Zaharia. Stereoscopic photos set.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE IMPORTANCE OF TREES IN URBAN ALIGNMENTS. STUDY OF VEGETATION ON KISELEFF BOULEVARD, BUCHAREST

Elisabeta DOBRESCU, Claudia FABIAN

University of Agronomic Sciences and Veterinary Medicine Bucharest,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: veradobrescu@gmail.com

Abstract

Tree alignment plays an important role in shaping the busy urban roadways. These types of trees play a crucial role in rendering ecosystem services, while, at the same time, imparting a characteristic image of the place. The sustainable management of the species used in these alignments as well as the process of replacing them with other species are two of the major issues which fall under the scope of specialists concerned with ensuring a high quality urban environment. In light of the constantly changing climatic factors and an increasingly polluted urban environment, the studies on the alignments from Kiseleff Boulevard give rise to discussions on the choice of the most suitable tree species for an urban area with high traffic. Moreover, the use of species must be done after a rigorous selection and after studying the local environmental conditions. Apart from that, in order to preserve the efficient ecosystem services, it is essential to properly manage the trees that are currently found in the previously mentioned alignments. The tree's health, its visual impact, the shading capacity, the age are just a few basic elements that shape and restore the image of a boulevard that is a landmark of the urban area of Bucharest. Dendrometrical studies have brought forward the direct link between the age and the state of the tree (its health) on the one hand and the ability to adapt of the studied species to the specific conditions of the Bucharest urban environment on the other.

Keywords*: Kiseleff Boulevard, urban environment, ecosystem services, dendrometrical studies.*

INTRODUCTION

Over the centuries, trees have had a great importance in the formation and shaping of the public space, sketching and giving life to promenades, alleys, avenues, squares, gardens and parks (Forrest and Konijnendijk, 2005). Planting trees in alignment in public spaces from the European area was one of the key elements in the cities in terms of building a certain image and raising the quality of urban areas.

If at the beginning, the trees were elements that contributed to the design and organization of the urban space, they've gradually become key elements of the site's identity. Depending on the geographical typologies, local and European influences, personal affinities or preferences, boulevards have always had various species in the alignment which provided a special image. Ever since the sixteenth century, European cities have paid attention to the trees, using them to mark the way to cathedrals and churches. One example is Paris where, during King Henry II of France, it was mandatory to plant and take care of trees, and also design and organize any other aligned planting of trees (Forrest and Konijnendijk, 2005).

Starting with the second half of the 19th century, promenades and boulevards from all around Europe transformed into favoured places where people gather to socialize. The indiscriminate popularity of the walks in these places revealed the vegetation's role in producing oxygen (Pellegrini, 2012), and the middle of the nineteenth century allowed the appearance of a true fact of society: healthy trees, sidewalk and pedestrians (Pellegrini, 2012). With the opening of walks for everybody, sidewalks are charged with the role of protecting pedestrians from carriages with horses and sidewalks are also the ones that will enable the introduction of tree alignment. One such example is the 19th century Paris during the transformation of the Haussmannien urbanism, when alignments of trees were made depending on three main characteristics: hygiene, comfort and aesthetics (Pellegrini, 2012). In the haussamannian Paris, the trees which most often encountered in road alignments are plane trees and chestnut trees, but also other species (Pellegrini, 2012) which could be found sporadically on avenues. Their planting was determined by the fashion of that time or certain preferences.

The situation in Bucharest proved to be similar to that of other European cities. Located in the northern part of Bucharest, the Kiseleff Boulevard is a historic roadway, which was built in the 1840s, at the same time with the urban changes Bucharest went through in the early nineteenth century (Fezi, 2010). Kiseleff represented the beginning of the road between Bucharest and Brasov, and at the same time the place outside the city where Mogoșoaia road ended (what we call Victoria Avenue after 1878). The documents which required the presence of this place where people were supposed to relax and socialize are the organic regulations (Toma, 2001). The place has been known since 1833 when the Great Steward (Romanian title assigned to the noble who was in charge of supervising the court) G. Filimon mentions it in a letter to General Kiselev. The alley was to be covered with sand and designed by "replanting the trees on the sides" (Toma, 2001). It was originally planted with *Tilia* sp. chosen for their shading capacity, and quickly enriched with *Robinia* sp. plantations. Thus, after 1846 Meyer proposes the extension of the *Tilia* sp. alley from Mogoșoaia road to the first round. From here until the second round (nowadays Arc de Triomphe) and further to Baneasa alley, it was overshadowed by an alignment of two rows of *Robinia pseudoacacia*. After 1851 the boulevard was extended to the third round (currently Press Square).

Due to the newly improvements that had been added to the street (mostly trees and gravel), it quickly became "the usual place of promenade" (Fezi, 2010) and of walking of the high society from Bucharest, offering opportunities to meet and socialize with other people, images which remained forever in the memory of the inhabitants (Toma, 2001). *Tilia* sp. were chosen as alignment trees and were planted in four rows. Their number was initially over 2000 (Vătămanu, 1973). Following the changes undertaken since the twentieth century, the boulevard increased and was enriched with new species of trees.

Based on these considerations, the study aims to establish the state of the inventoried trees, taking into account the fact that over time the trees used initially were replaced. If at the beginning the space chosen for this research was planted with *Robinia pseudoacacia* displayed in four rows, the boulevard contains nowadays alignments of *Fraxinus excelsior, Platanus hybrida, Aesculus hippocastanum, Tilia* sp., and other species used in urban areas now.

The role of the study is to present an inventory of plant material found in section A from the Press Square to the Arc de Triomphe and to establish after careful dendrometrical measurements the health of the inventoried species. This research started from the need to determine the steps to replace the species of trees after a certain period of time and also to find effective methods for using the species that will replace the damaged trees or those which are at the end of life.

The prevention of the physiological decline of the alignment leads to the maintenance of the urban image. Various studies have shown that trees which are in poor health affect the urban image and lead to a reduction of the space's social role and especially the ecological role.

MATERIALS AND METHODS

The inventory of species on Kiseleff Boulevard aimed trees throughout the entire chosen section, from Victory Square to the Press Square. This street is most certainly a historical landmark for urban transformation of the city. To improve the results of the research, the boulevard was divided into two sections. Section A from the Press Square to the Arc de Triomphe and section B from the Arc de Triomphe to Victory Square. But this article only regards section A, the remaining inventory thus contained in a larger study. The tree inventory was conducted using mixed teams made up of landscape architects and horticulture experts.

The working method in the inventory was to create sheets that contained both quantitative criteria such as: size, health, tree circumference at 1.3 m, but also qualitative criteria: aesthetic image, maintenance cutting or regeneration. The chosen qualitative and quantitative criteria

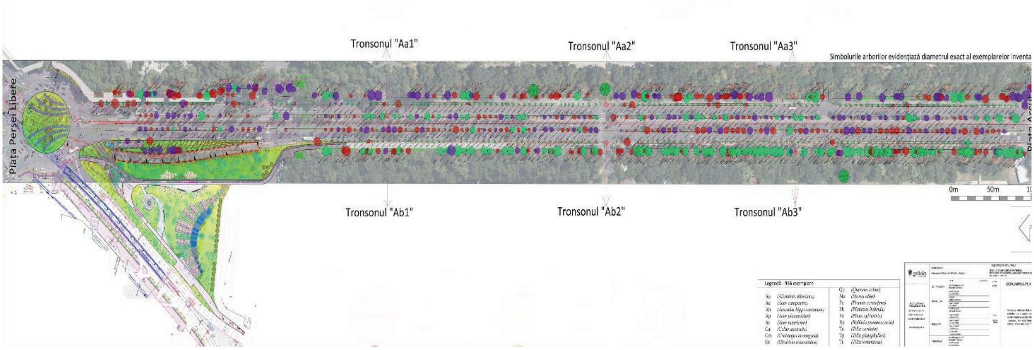


Figure 1. The section A of Kiseleff Boulevard

will complete the information about ecosystem services such as shading, carbon storage, storage of dust particles etc.

The cultural importance is also a criterion that is worth taking into account, because the species of trees from Kiseleff Boulevard have been replaced over time, and the newly planted species were not always adapted to Bucharest's urban environment. Thus, the selected criteria fall into four main groups:

- General features of the trees (the identification number of each tree, the circumference of the trunk, the trunk diameter, the insertion height of the treetop, the treetop diameter and the total height of the tree).
- The health of the tree (excellent, good, bad or very bad, dry) - which is determined only by the appearance of the tree and / or by determining diseases and pests that cause visible damage.
- The aesthetic value - relevant for determining the image of the boulevard as well as the possibility of decorating the chosen species. The argument for the introduction of this criterion is a qualitative one since cultural services are important for any recreation area. By determining aesthetic value, we can deduce the attractiveness or the monotony that can be induced by an alignment along a boulevard.

The maintenance status is justified by the need to intervene, types of interventions: cuts and treatments that can be performed on site depending on the chosen management plan.

The alignment referred to with the numeral 3 is actually the first line of trees which border the boulevard. In these generous green spaces, the number of trees and the number of trees species are various and bring an extra ecological value to the boulevard (Figure 1).

The chosen criteria are closely linked to the health of the trees, but also their aesthetic value, due to the fact that proper caring for urban trees provides an extension of their lives and also add value for the recreation component. For the inventory of the alignment of the avenue trees as well as a more careful study, each section was subdivided (Aa1, Aa2, Aa3 etc.) depending on the position of the alignment in the plan. Section A has four well established alignment sections.

RESULTS AND DISCUSSIONS

In the crowded cities where there are densely designed alignments, studies have shown that it is necessary to maintain and continuously restore a healthy environment to ensure a better quality of life. All this can be possible with the help of urban green spaces that, aside from their environmental qualities, help build a unique identity to the respective areas (Heidt and Neef, 2008).

If it base our research on the idea that trees play a crucial role in preserving a healthy environment, their importance is clear from both the absorption and storage of carbon dioxide and other polluting compounds, but also from the way they help reduce density of the ground by preventing flow (Merse et al., 2009). Planted near busy highways, trees can cut down the sound by reducing noise and pollution. It is known that small spaces with trees and shrubs can absorb or neutralize 68 tonnes of dust per ha / year and streets, squares and small parks with trees can absorb between

20% and 25% of the amount of dust that is found in the areas free of trees (Heidt and Neef, 2008). Thus, on green areas 50-100 m wide, they affect air quality up to a distance of 300 m (Heidt and Neef, 2008). Small spaces with trees (Merse et al., 2009), especially street alignment and boulevards cause a slower growth and lower life expectancy, especially when planted in crowded areas (Merse et al., 2009). The effects of urban pollution on vegetation and trees are the decrease of the default period of life, a slower development and an unattractive and sad appearance of the green elements. In the case of the Kiseleff Boulevard, trees have always played an important role for the spatial configuration and for providing ecosystem services. Its embellishment proved to be quite costly. The boulevard was in a continuous change over time (Potra, 1990). Thus, after the inventory conducted in mixed teams, section A with its subdivisions Aa1-Ab1, Aa2 - Ab2, Aa3 - Ab3 has a total of 956 specimens of 18 species out of which only one species of coniferous wood: *Pinus sylvestris*, the remaining 17 species belonging to the group of deciduous trees.
The middle layout Aa1 - Ab1, which has an alignment of *Fraxinus excelsior*, we have a number of 283 trees and for Aa2 and Ab2 *Platanus hybrida* alignments, with a total of 324 trees. In the case of Aa3 and Ab3 alignments, where we took stock of the trees from the first row, their total number is 349 (Figure 2).

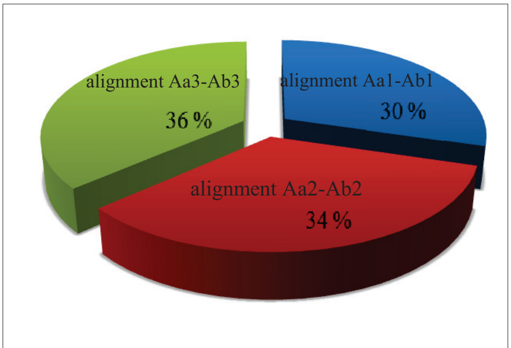


Figure 2. Total of the trees of the section A

As a result of the analysis of the three types of alignment, it was discovered that the diversity of species and volumes is mostly found in the boulevard's sides, in the portions numbered Aa3 and Ab3.
The alignment Aa1 - Ab1 has 283 tress, out of which 278 are *Fraxinus excelsior* and only 5 specimens of *Platanus hybrida*. In percentage, for a clearer image of the make-up of the alignments, 98.23% are part of the *Fraxinus excelsior* and the remaining 1.77% trees are *Platanus hybrida*. Note that this middle alignment consists of trees that have almost reached maturity, a result of the measurements carried out. Therefore, depending on the height of the specimens they may be classified into three distinct categories 2 m – 6 m (23%), 7 m - 9.5 m (39%) and 10 - 12 m (38%).
Following evaluation, it has been determined that out of all trees from the species *Fraxinus excelsior*, a percentage of 17.67% are in a state of good health, while the majority of specimens, 45.58% are in a mediocre state of health. Trees with a poor health condition or even dried specimens are found at a rate of 36.74%. This means that for more than one third of the inventoried specimens, more frequent cutting actions need to be applied to allow them to regenerate and recover. The state of the trees is a reflection of the way we manage all maintenance processes as well as a picture of the way trees and other plants are chosen and whether they are adequately matched to the environmental conditions and requirements.
But no species is totally resistant to pollution, because pollution resistance can be determined by several indicators that are constrained by certain characteristics of pollutants, the plant's growth stage, health status, location (Sieghardt et al., 2005).
For the following two alignments Aa2 and Ab2, the dominant species is *Platanus hybrida*. It must be noted that the alignment Aa2 is made up mostly of *Platanus hybrida* trees (88%). Also, in this alignment, *Fraxinus excelsior* is located in a proportion of 10%, as well as other species 2%. There are 154 trees on the alignment Aa2 that are between 4 meters and 7.5 meters high and only 17 trees between 8 and 16 meters high. 96% of them are young trees (148 trees) in good condition and only 4% are not in a very good condition or they are dry. The majority (70%) of the 17 trees between 8 meters and 16 meters high are in poor

conditions or very dry and only 6% are in good health.
It is easy to observe that most trees of *Platanus hybrida* are young specimen that were planted recently (the year of planting is usually 2013). It appears that trees are becoming increasingly fragile and their health status changes as they adapt increasingly difficult to urban pollution and its consequences.
In alignment Ab2 among the 153 trees we find species of *Platanus hybrida* - 145 trees with heights between 4 meters and 7 meters, 5 trees *Fraxinus excelsior* with heights of 8 meters and 12 meters and 3 specimens of *Aesculus hippocastanum* heights of 8 meters and 10 meters. Specimens of *Platanus hybrida* and those of *Aesculus hippocastanum* are young trees, while those of *Fraxinus excelsior* have almost reached maturity.
The health criteria reflects about the same situation as in alignment Aa2. The young species of *Platanus hybrida* (145 trees) are in good health, being planted throughout 2013, but specimens of *Fraxinus excelsior* and *Aesculus hippocastanum* are in bad health condition. Again, we can conclude that at the time of this study, namely in 2015, the majority of trees from the alignments Aa2 and Ab2 are newly planted ones and that they are trying to adapt to a boulevard with dense traffic conditions. But as they get older, their health deteriorates as well.
In section A, the last subdivisions Aa3 and Ab3 are actually the first lines of trees from the massive edges which complete this avenue's image. The trees inventoried in both Aa3 and Ab3 are mostly quite diverse, part of a large number of species and are found either in good health or in a bad condition.
Thus in Aa3, there are 175 trees of 16 of hardwood as *Acer* sp., *Tillia* sp., *Robinia pseudoacacia* etc.
For the Ab3 alignment, most of the 171 trees are *Tillia* sp., *Fraxinus excelsior* and *Aesculus hippocastanum*, but with a higher procentage regarding their condition (70% of them are in a good health state).
One of the results of the study show that the diversity encountered in certain areas has helped the adaptation process of the trees and generally speaking, the majority of them has reached maturity in good conditions.
An important role in urban areas is played by this diversity of species which helps to create and maintain a stable urban environment. Therefore species diversity in urban ecosystem management is one of the most important elements that should been encouraged as it is desirable to have a balance between biological diversity and production and maintenance costs of the plants (Heidt and Neef, 2008).
Depending on the adaptability of each tree, there could be a significant growth rate in the first years. However, there could also be trees which grow more slowly after they were planted and then, in a few years, they dry out and die (Bradshaw et al., 1995). Therefore, in the case of the species used for alignment, the criteria behind their management refers primarily to the possibility of survival and the selection of the best adapted trees, depending on the site's characteristics (Heidt and Neef, 2008). Managing the trees on Kiseleff Boulevard, section A, which was presented in this article also provides an updated and correct choice of tree species that are most suited to the conditions of urban pollution. The study observed that age and health are related and that most mature specimens identified at the site are in a poor state of health. This situation is caused not only by the characteristics of the urban environment, but also by the poor management from stakeholders.

CONCLUSIONS

It is therefore necessary to establish a vegetation management plan to anchor the current conditions of a polluted urban environment. Newly taken actions on trees alignment in European cities seek a closer study of the relationship between the area where the tree will be planted and the stresses to which specimens are exposed. It has been found that the life of trees is shorter in urban areas because of the high stress levels they face (Sæbø et al., 2005). Experts have identified many stress factors: from pollutants, physical damage, excessive cuts of treetops and the very small space for root development, to a soil that is poor in nutrients and is unable to absorb the water and oxygen needed for the roots (Sæbø et al., 2005).

For a better determination of the species which are well adapted to a particular local context, one can use the numerous studies that rely both on species selection and a more careful selection of genotypes. So the selection of urban trees is made after three criteria determining: the species' adaptation to environmental conditions of the site, the way its ecological functions adapt to urban conditions and a low cost of production and management (Sæbø et al., 2005).

The trees from street alignments must have several physical characteristics that recommend them for this type of use: they must have a strong growth of ramifications, well defined branches, with a steady and predictable growth and with a long life in order to lower or remove successive costs and a certain aesthetic value (Sæbø et al., 2005).The present study showed that a large proportion of all inventoried trees reach maturity in a mediocre state of health.

A tree that is in a poor or mediocre health state loses its environmental, social, cultural and economic benefits (Bradshaw et al., 1995). Therefore, encouraging a quality management which has among its concerns urban trees is desirable. Selecting and managing tree by tree translates into a better quality of life and reduction of urban pollution

REFERENCES

Bradshaw A.D., Hunt B., Walmsley T.J., 1995. Trees in the Urban Landscape: Principles and practice, Ed. University Press, Cambrige, 17-22.

Fezi B.A., 2010. Bucurestiul European, Ed. CurteVeche Publishing, București, 49-53.

Forrest M., Konijnendijk C., 2005. A History of Urban Forests and Trees in Europe, in "Urban Forests and Trees: A Reference Book" by Konijnendijk, C.C., Nilsson, K., Randrup, Th.B., Schipperijn, J., Ed. Springer Science & Business Media, Verlag, Berlin, Heidelberg, 23-48.

Heidt V., Neef M., 2008. Benefits of Urban Green Space for improving urban climate, in Ecology, Planning, and Management of Urban Forests. International Perspectives for Improving Urban Climate, by Carreiro, M.,M., Song, Y.-C., Wu, J., Springer Science+Business Media, New York, 84-96.

Merse C., Buckley G.L., Boone C.G., 2009. Street trees and urban renewal: a Baltimore case study, in The Geographical Bulletin, vol.50(2):65-81.

Pellegrini P., 2012. Pieds d'arbre, trottoirs et piétons : vers une combinaison durable?, Développement durable et territoires, vol.3(2):1–16.

Potra G., 1990. Din Bucureștii de ieri, Ed. Științifică și Enciclopedică, București, 329-334.

Sæbø A., Borzan Z., Ducatillion C., Hatzistathis A., Lagerström T., Supuka J., García-Valdecantos J.L., Rego F., Slycken J. V., 2005. The Selection of Plant Materials for Street Trees, Park Trees and Urban Woodland, in "Urban Forests and Trees: A Reference Book" by Konijnendijk, C.C., Nilsson, K., Randrup, Th.B., Schipperijn, J., Ed. Springer Science & Business Media, Verlag, Berlin, Heidelberg, 257-280.

Sieghardt M., Mursch-Radlgruber E., Paoletti E., Couenberg E., Dimitrakopoulus A., Rego F., Hatzistathis A., Randrup T.B., 2005. The Abiotic Urban Environment: Impact of Urban Growing Conditions on Urban Vegetation, in "Urban Forests and Trees: A Reference Book" by Konijnendijk, C.C., Nilsson, K., Randrup, Th.B., Schipperijn, J., Ed. Springer Science & Business Media, Verlag, Berlin, Heidelberg, 281-324.

Toma D., 2001. Despre grădini și modurile lor de folosire, Ed. Polirom, Iași, 144-164.

Vătămanu N., 1973. Istorie bucureșteană, Editura Enciclopedica Romana, București, 104-109.

MISCELLANEOUS

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECT OF SECONDARY METABOLITES PRODUCED BY DIFFERENT *TRICHODERMA* SPP. ISOLATES AGAINST *FUSARIUM OXYSPORUM* F.SP. *RADICIS-LYCOPERSICI* AND *FUSARIUM SOLANI*

Cristina PETRIȘOR, Alexandru PAICA, Florica CONSTANTINESCU

Research and Development Institute for Plant Protection,
Ion Ionescu de la Brad Blvd., No.8, District 1, Bucharest, Romania

Corresponding author email: crisstop@yahoo.com

Abstract

Secondary metabolites produced by filamentous fungi have different structure and function, and they are a source of novel compounds with pharmaceutical, agricultural and medicinal importance. Trichoderma spp. are considered to be an abundant source of secondary metabolites, some of them with applications in biological control, plant growth promotion, like aroma constituents or in plant immunity. The aim of this study was to assess the potential of volatile and non-volatile metabolites released from some antagonistic Trichoderma spp. isolates against pathogens Fusarium oxysporum f.sp. radicis-lycopersici and Fusarium solani which causes wilting for more cultures. In vitro studies have demonstrate that volatile compounds produced by T49 (67.69%), Tk14 (64.61%), T50 (61.53 %), T85 (60%) showed strong inhibitory effect on FORL growth compared with M14 isolate. The non-volatile tests revealed that three isolates of Trichoderma (T85, T50, Tal12) are the best which inhibited the growth of F.solani in vitro.

Key words: *volatile compounds, non-volatile compounds, biocontrol, antifungal effects.*

INTRODUCTION

Fusarium sp. is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, is considered as one of the main soil-borne systemic diseases and the major limiting factor in the production of tomato both in greenhouse and field-grown (Srivastava et al., 2010; Borrero et al., 2004).

Various species of fungi described as antagonists of phytopathogenic fungi produce secondary metabolites with strong antifungal activity. *Trichoderma* species have been used widely as biocontrol agents because produce many antifungal metabolites, volatile and non-volatile that adversely affect growth of different fungi phytopathogens (Li et al., 2016; Barakat et al., 2014; Nagendra et al., 2011; Ajith and Lakshmidevi, 2010; Srivastava et al., 2010; Faheem et al., 2007; Vinale et al., 2006; Dennis and Webster 1971a; Dennis and Webster 1971b). Also, the potential of *Trichoderma* spp. to produce many volatile (e.g. pyrones, sesquiterpenes) and non-volatile secondary metabolites (e.g.) has been reviewed by Reino et al., 2008. *Trichoderma* spp. differ in their abilities to produce volatile and non-volatile secondary metabolites and their production varies greatly between species and between isolates of the same species, depends on environmental conditions (Vinale et al., 2009).

Many previous studies revealed that antimicrobial metabolites produced by *Trichoderma* spp. are effective against a wide range of phytopathogenic fungi, *Botrytis fabae*, *Fusarium* spp, *Rhizoctonia solani*, *Macrophomina phaseolina* (Chen et al., 2015; Barakat et al., 2014; Sreedevi et al., 2011).

Dubey et al., 2011 reported that secondary metabolites from culture filtrates with higher concentration of *Trichoderma viride, Trichoderma virens and Trichoderma harzianum* inhibited mycelial growth of *Fusarium oxysporum* f.sp. *ciceris*. Vinale et al., 2006 reported that volatile secondary metabolites play a key role not only in mycoparasitism by *Trichoderma harzianum* and *Trichoderma atroviride*, but also in their interactions with tomato and canola seedlings. Ajith and Lakshmidevi, 2010 reported the effect of volatile and non-volatile compounds produced by *Trichoderma* spp. against *Colletotrichum capsici*, a fungal pathogen responsible for anthracnose disease in bell peppers. The results of this authors showed that the volatile compounds produced by *Trichoderma* spp.

showed 30 to 67% inhibition of *Colletotrichum capsici,* whereas non-volatile compounds have the ability to control growth of *Colletotrichum capsici* by 21 to 68% at a concentration of 50% culture filtrate. In Romania there is little information available on the implication of secondary metabolites produced by *Trichoderma* with inhibitory effect on different pathogens (Raut et al., 2014a; Raut et al., 2014b).

The aim of the present study was to determine, *in vitro*, effect of volatile and nonvolatile compounds produced by some *Trichoderma* isolates against fungal plant pathogens such as *Fusarium oxysporum* f.sp. *radicis-lycopersici* and *Fusarium solani.*

MATERIALS AND METHODS

The tested *Trichoderma* isolates as well as phytopahogens *Fusarium oxysporum* f.sp. *radicis-lycopersici (FORL)* and *Fusarium solani* (*F.solani*) used in this experiment belong to Microbial Collection of RDIPP.

Effect of volatile compounds produced by Trichoderma isolates on the mycelial growth of Fusarium

The effect of volatile metabolites produced by *Trichoderma* against *Fusarium* was tested using the inverted plate technique described by Dennis and Webster, 1971a. The mycelial disk (5 mm) of *Trichoderma* excised from the edge of 5 days old cultures was inoculated into the center of a Petri dish which containing PDA medium. The lid of each plate was replaced by the bottom of a plate containing PDA medium inoculated with a 5-mm-diameter mycelial disk of *FORL and F solani* so as test pathogens were directly exposed to antagonistic environment created by *Trichoderma*. Then, the two plates were sealed together with parafilm and incubated at 28°C for 6 days in the dark. The control sets did not contain the antagonist. Radial growth of pathogens was recorded after 6 days of incubation and percentage inhibition was calculated in relation to control.

Effect of non-volatile compounds produced by Trichoderma isolates on the mycelial growth of Fusarium

The production of non-volatile compounds by *Trichoderma* isolates against *Fusarium* was studied using the method described by Dennis and Webster (1971b). Initially, mycelia agar plugs (5mm diameter) removed from the edge of a 5 days old *Trichoderma isolates* mycelium were inoculated in 100 ml sterilized Potato Dextrose Broth in 250 ml conical flasks, and incubated at 28±2°C on a rotatory shaker set at 100 rpm for 10 days. The culture filtrate was filtered through Whatman paper for removing mycelial mats and then centrifugated at 3000 rpm 10 minutes.

The filtrate was added to molten PDA medium (at 40±3°C) to obtain a final concentration of 10% (v/v), 25% (v/v), 50% (v/v). Then PDA containing Petri dishes were inoculated with mycelial plugs (5 mm diameter) of *FORL* and *F. solani* at the centres. The dishes were incubated at 26±2°C until the colony reached the plate edge. Plates without filtrate served as control. There were three replicates for each treatment and the experiment was repeated two times. The percentage inhibition was calculated in relation to the control by the formula:

$$L=C-T/C\times100$$

where: L – inhibition of radial mycelial growth; C – the radial growth measurement of the pathogen in the control; T– the radial growth measurement of the pathogen in the presence of antagonists.

RESULTS AND DISCUSSIONS

A large variety of volatile secondary metabolites could be produced by *Trichoderma* spp. such as ethylene, hydrogen cyanide, aldehydes and ketones, which play an important role in controlling various plant pathogens (Faheem et al., 2010; Siddique et al., 2012; Chen et al., 2015). The results for volatile metabolites activity against pathogens are presented in table 1 and figure1.

From our results, it is evident that volatile compounds produced by *Trichoderma* isolates studied decreased the mycelial growth of *FORL* and *F.solani* (table 1). The effects of volatiles produced by the *Trichoderma* isolates studied over the 6-day incubation period were different for *FORL* and *F. solani*. *In vitro* studies showed that the volatile compounds produced by T49, T50, Tk14, T85 and Tal12 significantly reduced mycelial growth and inhibited spore germination of *FORL* with inhibition percent between 58.46% and 67.69%.

Table1 Effect of volatile compounds produced by different *Trichoderma* strains on *Fusarium* mycelial growth

Pathogen	Antagonist *Trichoderma* strain	Growth inhibition (%)
Fusarium oxysporum f.sp. *radicis-lycopersici*	T50	61.53
	T49	67.69
	T85	60
	Tal12	58.46
	Tk14	64.61
	TK20	56.15
	M14	31.53
Fusarium solani	T50	55.38
	T49	32.30
	T85	33.84
	Tal12	66.15
	Tk14	48.76
	TK20	38.46
	M14	29.23

Volatile metabolites produced by Td49 were more efficient in reducing the mycelial growth of *FORL* by 67.69%, after 6 days of incubation, respectively than M14 being 31.53%. The data obtained show that the Tal12 (66.15%), T50 (55.38%) and Tk14 (48.76%) strains producing volatile compounds with significant effect in reducing growth of phytopatogenic fungi *F.solani.* Other strains studied (T49, T85, Tk20, M14) inhibit very weak the growth of *F.solani,* inhibition percentages ranging from 29.23% (M14) to 38.46% (Tk20).The high degree of growth inhibition of *FORL* by all of the strains suggests that the inhibitory effect observed in dual culture could mostly be attributed to volatile metabolites.

Many strains of *Trichoderma* have been reported to produce volatile compounds that inhibit the growth of pathogen fungi significantly. Studies of Zhang et al. (2014) showed that the volatile compounds produced by *Trichoderma harzianum* T-E5 have an significant inhibition of *FOC* mycelial growth but not by killing *FOC*. This investigation suggests that metabolites released by these *Trichoderma* species are toxic and fungistatic to *Fusarium.*

Some *Trichoderma* species (*T. viride* and *T.asperellum*) tested by Qualhato et al., (2013) produced volatile metabolites having significant effects on the mycelial growth and development of the *S. sclerotiorum*, *F.solani.*

Tapwal et al. (2011) reported that volatile compounds of *Trichoderma* spp. isolates significantly inhibited the mycelial growth and spore germination of *F.oxysporum.*

Research of Calistru et al. (1997) revealed that volatile metabolites produced by *Trichoderma harzianum* species can significantly suppress the growth of *Aspergillus flavus and Fusarium moniliforme* rather than mycoparasitism. Also, studies of Raut et al. (2014a) demonstrated that volatile metabolites produced by two *Trichoderma* strains displayed inhibitory effects on *R. solani* and *P.ultimum* pathogens growth.

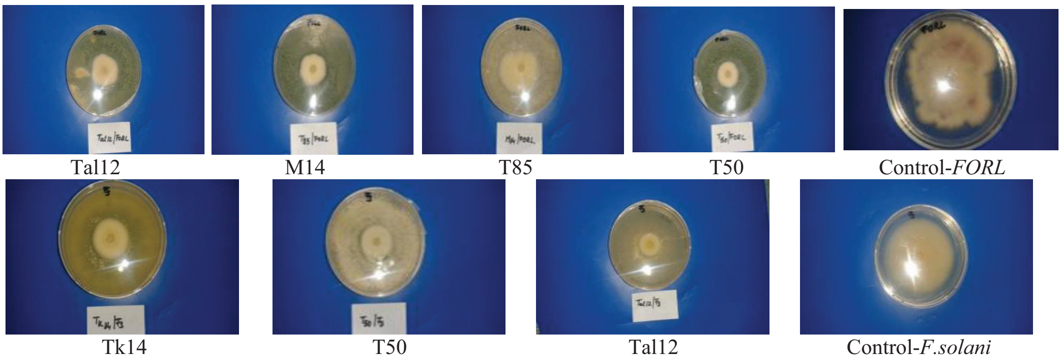


Figure 1. Plate assay for the influence of volatile metabolites from *Trichoderma* isolates on the mycelial growth of fungal pathogens

The effect of 10%, 25%, 50% filtrate concentration of *Trichoderma* isolates on *F. solani* radial growth has been show in figure 2. All concentrations of the metabolites reduced radial growth of *F.solani* in different percent.T50 and T85 and Tal12 isolates showed the highest inhibition of *F.solani* radial growth, however Tk20 and M14 had the lowest effect on growth of this pathogen. The efficiency of the non-volatile metabolites on the mycelial growth of the pathogenic strain of *F.solani* varied from 30% and 70%. This results are in accordance with of Raut et al., 2014b that supported that 25% and 50% filtrate concentration of *Trichoderma* isolate produced between 70-80% inhibition of *F.solani.*

The non-volatile secondary metabolites produced by *Trichoderma* isolates used in this study were found to be more effective in suppressing the mycelia growth of *Fusarium solani* when compared to FORL.

Our results is consistent with the results of Kavitha and Nelson, 2013 which supported that non-volatile compound of *Trichoderma* inhibited growth of *Fusarium javanicum* and *Fusarium oxysporum.* This results is supported by the previous reports of Hasan et al., 2012 which found that *Trichoderma harzianum* inhibited the radial growth of *Fusarium graminearum* by 43.33%.

The concentrated solutions (50%) of the *Trichoderma* culture filtrates suppressed the growth of *FORL* but weaker compared to *F.solani*. Neverthelss culture filtrates of *Trichoderma* diluted (10%, 25%) showed very low inhibition level (20%) of this pathogen.

Some investigations suggest that metabolites released by *Trichoderma* species are the most effective on *Fusarium culmorum* and can be used successfully to control *Fusarium* foot rot in wheat seedlings (El-Hasan et al., 2008). Also, Barakat et al. (2014) founded that the non-volatile secondary metabolites of *Trichoderma* species were more effective in suppressing the mycelial growth of *Botrytis fabae* when compared to volatile compound.

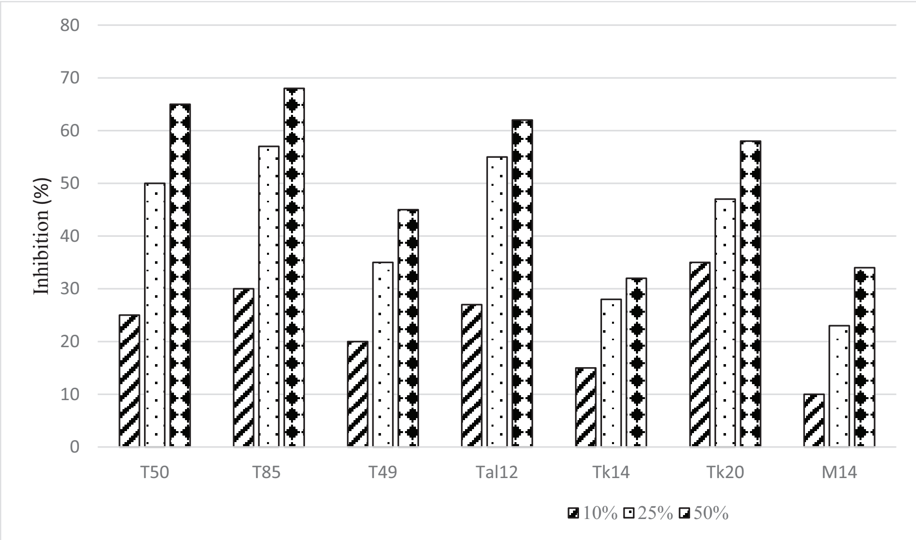


Figure 2. Effect of different concentrations of non-volatile metabolites produced by *Trichoderma* isolates on radial growth of *Fusarium solani* after 6 days of inoculation

CONCLUSIONS

Our results demonstrated the involvement of volatile and non-volatile compounds in the inhibition of *FORL* and *F.solani*

All *Trichoderma* isolate produced volatile compounds having significant effect in reducing the growth of *Fusarium.*

The volatile compounds produced by T49, T50, T85 and Tk14 isolates significantly reduced the radial growth of *FORL*.

The non-volatile compounds produced by filtrate concentrations (25% and 50%) of T85, T50 and Tal12 inhibited between 50-70% radial growth of *F.solani.*

The present results showed that the ability of secondary metabolites production is different among isolates studied .

ACKNOWLEDGEMENTS

This paper is supported by Ministry of Research and Innovation through Core Programme and was financed from the project PN 16 29- 02-01/2016.

REFERENCES

Ajith P.S., Lakshmidevi N., 2010. Effect of volatile and non-volatile compounds from *Trichoderma* spp against *Colletotrichum capsici* incitant of anthracnose on bell peppers. Nature and Science, 8(9): 265-269.

Barakat F. M., Abada K. A., Abou-Zeid N. M., El-Gammal Y. H. E., 2014. Effect of volatile and non-volatile compounds of *Trichoderma* spp. on *Botrytis fabae* the causative agent of faba bean chocolate spot. American Journal of Life Science, 2(6-2):11-18.

Borrero C., Trillas M.I., Ordovas J., Tello J.C., Aviles M., 2004. Predictive factors for the suppression of *Fusarium* wilt of tomato in plant growth media. Phytopathology, 94:1094-1101.

Calistru C., McLean M., Berjak P., 1997. *In vitro* studies on the potential for biological control of *Aspergillus flavus* and *Fusarium moniliforme* by *Trichoderma* species: A study of the production of extracellular metabolites by *Trichoderma* species. Mycopathologia, 137: 115-124.

Chen L.J, Sun S.Z., Miao C.P, Wu K., Chen Y.W., Xu L.H., Guan H.L., Zhao L.X., 2015. Endophytic *Trichoderma gamsii* YIM PH 30019: a promising biocontrol agent with hyperosmolar, mycoparasitism and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of *Panax notoginseng*. Journal of Ginseng Research, 38:1-10.

Dennis C., Webster J., 1971a. Antagonistic properties of species groups of *Trichoderma* II. Production of volatile antibiotics.Trans. Br. Mycol. Soc., 57: 41-48.

Dennis C., Webster J., 1971b. Antagonistic properties of species groups of *Trichoderma* I. Production of non-volatile antibiotics. Trans. Brit. Mycol. Soc., 57: 25-39.

Dubey S.C., Tripathi A., Dureja P., Grover A., 2011. Characterization of secondary metabolites and enzymes produced by *Trichoderma* species and their efficacy against plant pathogenic fungi. Indian J Agr. Sci., 81:455–461.

El-Hasan A., Walker F., Buchenauer H., 2008. *Trichoderma harzianum* and its metabolite 6-pentyl-alpha-pyrone suppress fusaric acid produced by *Fusarium moniliforme*. J. Phytopathol,156: 79-87.

Faheem A., Razdan V.K., Mohiddin F.A., Bhat K.A., Sheikh P.A., 2010. Effect of volatile metabolites of *Trichoderma* spp. against seven fungal plant pathogens *in vitro*. J. Phytol, 2(10): 34-3.

Kavitha T., Nelson R., 2013. Exploiting the biocontrol activity of *Trichoderma* spp against root rot causing phytopathogens. ARPN Journal of Agricultural and Biological Science, 8(7):571-574.

Li Y., Sun R., Yu J., Saravanakumar K., Chen J., 2016. Antagonistic and Biocontrol Potential of *Trichoderma asperellum* ZJSX5003 against the maize stalk rot pathogen *Fusarium graminearum*. Indian J Microbiol, 56(3):318–327.

Nagendra B., Prasad A., Kumar M.R., 2011. Effect of non-volatile compounds produced by *Trichoderma* spp. on growth and sclerotial viability of *Rhizoctonia solani*, incitant of sheath blight of rice. Indian J. Fundamental Appl. Life Sci,1 (2) 37-42.

Qualhato F.T., Cardoso-Lopes F.A., Steindorff A.S., Brandao R.S., Amorim Jesuino S.R., Ulhoa C.J., 2013. Mycoparasitism studies of *Trichoderma* species against three phytopathogenic fungi: evaluation of antagonism and hydrolytic enzyme production Biotechnol. Letters, 35:1461–1468.

Reino J.L., Guerrero R.F., Hernandez-Galan R., Collado I.G., 2008. Secondary metabolites from species of the biocontrol agent *Trichoderma*. Pytochemistry Review, 7: 89–123.

Raut I., Doni-Badea M., Calin M., Oancea F., Vasilescu G., Sesan T., Jecu L., 2014a. Effect of volatile and non-volatile metabolites from *Trichoderma* spp. against important phytopathogens. Revista de Chimie, 65(11):1285-1288.

Raut I., Calin M., Vasilescu G., Doni-Badea M., Sesan T., Jecu L., 2014b. Effect of non volatile compounds of *Trichoderma* spp against *Fusarium graminearum, Rhizoctonia solani* and *Pythium ultimum*. Scientific Bulletin. Series F. Biotechnologies, Vol. XVIII 178-181.

Siddiquee S., Cheong B.E, Taslima K., Kausar H., Hasan M.M., 2012. Separation and identification of volatile compounds from liquid cultures of *Trichoderma harzianum* by GC-MS using three different capillary columns. Journal of Chromatographic Science 50:358-367.

Sreedevi B., Devi C., Saigopal D.V.R., 2011. Isolation and screening of effective *Trichoderma* spp. against the root rot pathogen *Macrophomina phaseolina* Journal of Agricultural Technology, 7(3): 623-635.

Srivastava R., Khalid A., Singh U.S., Sharma A.K., 2010. Evaluation of arbuscular mycorrhizal fungus, Fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. Biol. Control, 53:24-31.

Tapwal A., Singh U., Teixeira de Silva J.A., Singh G., Grag S., Kumar R., 2011. *In vitro* antagonism of *Trichoderma viride* against five phytopathogens. Pest Technology, 5 (1): 59–62.

Vinale F., Ghisalberti E.L., Sivasithamparam K., Marra R., Ritieni A., Ferracane R., Woo S., Lorito M., 2009. Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. Letters in Applied Microbiology, 48:705–11.

Vinale F., Marra R., Scala F., Ghisalberti E.L., Lorito M., Sivasithamparam K., 2006. Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. Lett Appl. Microbiology, 43:143–148.

Zhang F., Yang X., Ran W., Shen Q., 2014. *Fusarium oxysporum* induces the production of proteins and volatile organic compounds by *Trichoderma harzianum* TE-5. FEMS Microbiol.Lett.359:116-123

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ANTAGONISTIC ACTIVITY OF INDIGENOUS *PSEUDOMONAS* ISOLATES AGAINST *FUSARIUM* SPECIES ISOLATED FROM ANISE

Aleksandra STANOJKOVIĆ-SEBIĆ[1], Snežana PAVLOVIĆ[2], Mira STAROVIĆ[3], Radmila PIVIĆ[1], Zoran DINIĆ[1], Zorica LEPŠANOVIĆ[4], Dragana JOŠIĆ[1]

[1]Institute of Soil Science, Teodora Drajzera 7, 11000 Belgrade, Serbia
[2]Institute for Medicinal Plant Research “Dr Josif Pančić”, 11000 Belgrade, Serbia
[3]Institute for Plant Protection and Environment, 11000 Belgrade, Serbia
[4]Military Medical Academy, 11000 Belgrade, Serbia

Corresponding author email: astanojkovic@yahoo.com

Abstract

Fusarium species are widely distributed and responsible for several plant diseases in different medicinal plants. Fungi of this genera cause very important economic losses in Serbian plantation. Antibiotic production by plant-associated microorganisms represents an environmentally acceptable method of disease control, esspecialy in cultivation of medicinal and aromatic plants. Among the plant growth promoting bacteria (PGPB), Pseudomonas have been recognized as the most frequent antagonists of plant fungal pathogens and antibiotic producers. This is probably due to the widely distribution of this diverse group of bacteria in temperate soils and their often predomination among bacteria from plant rhizosphere. In this study, we examined the antifungal activity of eleven indigenous Pseudomonas isolates (PB4, PB5, K38, Q34, PBA12, PD5, C7, C8, Q16P, K29 and K35) against eight phytopathogenic fungi belonging to genus Fusarium (Fusarium tricinctum, F. sambucinum, F. equiseti, F. heterosporum, F. sporotrichioides, F. semitectum, F. verticillioides and F. oxysporum), which had infected anise (Pimpinella anisum L., fam. Apiaceae), using in vitro growth inhibition tests. The obtained results demonstrated that all Pseudomanas isolates showed more or less pronounced antifungal activity, whereby the most pronounced activity was observed for K29 and K35 strains. F. oxysporum and F. verticillioides showed the highest sensitivity to antibiotic-producing Pseudomanas isolates. In general, it has been concluded that studied Pseudomonas isolates have potential in controlling plant diseases caused by Fusarium spp., whereby the bacterial isolates with the highest inhibitory potential will be selected for further experiments.

Key words*: Pseudomonas, Fusarium spp., Pimpinella anisum, antifungal activity.*

INTRODUCTION

The use of chemical fertilizers and pesticides has caused an incredible harm to the environment. These agents are both hazardous to animals and humans and may persist and accumulate in natural ecosystems. An answer to this problem is replacing chemicals with biological approaches, which are considered more environment friendly in the long term. One of the emerging research area for the control of different phytopathogenic agents is the use of biocontrol plant growth promoting rhizobacteria (PGPR), which are capable of suppressing or preventing the phytopathogen damage (Nihorembere et al., 2011).

Phytopathogenic fungi, as the most common plant pathogens, are capable of infecting different types of plant tissues. Among the main aims in agriculture is finding adequate strategies for their suppression. One of these strategies is biological control (biocontrol) of plant diseases that relies on the use of natural antagonists of phytopathogenic fungi (Heydari and Pessarakli, 2010).

A special place among the natural antagonists of phytopathogenic fungi belongs to rhizobacteria that show beneficial effects on plant growth (PGPR) (Zehnder et al., 2001). These bacteria use various mechanisms for their action: production of plant hormones, asymbiotic fixation of N_2, antagonism towards phytopathogenic microorganisms and the ability to solubilize mineral phosphates and other nutrients (Cattelan et al., 1999). Different isolates of fluorescent *Pseudomonas* species take prominent place in this respect.

Consequently, these isolates have been intensively studied.
Fluorescent *Pseudomonas* species are present in temperate and tropical soils, often dominant among rhizobacteria (Ayyadurai et al., 2007). They belong to PGPR because of the ability to colonize the roots of plants and stimulate growth by decreasing the frequency of diseases. Suppression of diseases includes the inhibition of pathogens by competition and/or by antagonism (Couillerot et al., 2009). The prominent feature of fluorescent *Pseudomonas* species is the production of antibiotics as inhibitory compounds that play a role in the suppression of diseases caused by phytopathogenic fungi (Haas and Défago, 2005). One of the best-studied antibiotics of fluorescent *Pseudomonas* species are phenazines, nitrogen-containing heterocyclic compounds (Fernando et al., 2005). The only known natural producers of phenazines are bacteria (Pierson III and Pierson, 2010).
Fluorescent *Pseudomonas* species are capable of inhibiting the phytopathogenic fungi that belong to genus *Fusarium* (Showkat et al., 2012). *Fusarium* spp. are a widespread cosmopolitan group of fungi and commonly colonize aerial and subterranean plant parts, either as primary or secondary invaders. Some species are common in soil and it is rare to find necrotic root of a plant in most agricultural soils that is not colonized by at least one *Fusarium* sp. (Nelson et al., 1983).
One of the hosts of *Fusarium* spp. is anise (*Pimpinella anisum* L., fam. Apiaceae). Anise is an aromatic plant which is used in traditional medicine (especially its fruits) as carminative, aromatic, disinfectant and galactagogue (Shojaii and Abdollahi Fard, 2012).
The aim of this study was to examine the antifungal activity of eleven indigenous *Pseudomonas* isolates against the eight phytopathogenic fungi belonging to genus *Fusarium*, which had infected anise (*Pimpinella anisum* L., fam. Apiaceae).

MATERIALS AND METHODS

The antifungal activity of the following indigenous *Pseudomonas* isolates: PB4, PB5, K38, Q34, PBA12, PD5, C7, C8, Q16P, K29 and K35, was examined against the phytopathogenic fungi belonging to genus *Fusarium* (*F. oxysporum*, *F. tricinctum*, *F. sambucinum*, *F. equiseti*, *F. heterosporum*, *F. sporotrichioides*, *F. semitectum*, *F. verticillioides*), which had infected anise (*Pimpinella anisum* L., fam. Apiaceae).
The examination was conducted on Waksman agar plates nutrient media, using *in vitro* inhibition tests. Overnight cultures of the tested *Pseudomonas* isolates, optimized to $1 \cdot 10^7$ cfu/ml were used to examine the influence of extracellular metabolites of cells (1 ml of cultures was centrifuged at 13000 rpm for 10 min and resuspended in the same volume of sterile saline solution).
Inoculation of Waksman nutrient media with the tested cultures of *Pseudomonas* isolates was done near the edges of Petri dishes and mycelia of the studied *Fusarium* species were placed in the center. Control variants contained only mycelia of *Fusarium* species on Waksman agar plates.
Observation and the measuring of zones of growth inhibition of mycelia around bacterial colonies were performed after seven days of incubation at 25°C (Nair and Anith, 2009). The percentage of growth inhibition of mycelia of *Fusarium* species was calculated by the formula: % Inhibition = [(Control - Treatment)/Control] x 100 (Ogbebor and Adekunle, 2005).

RESULTS AND DISCUSSIONS

Due to the soil-borne nature of the diseases caused by *Fusarium* species the use of chemical methods for the control of disease is rarely successful. Inconsistencies in biocontrol under varying environmental conditions have been a common limitation of soil-borne pathogens. The present research was conducted to evaluate the efficacy of indigenous *Pseudomonas* isolates against these pathogens.
Table 1 displays the data on *in vitro* antifungal activity of selected *Pseudomonas* sp. isolates toward *Fusarium* species, which had infected anise.
The obtained results imposed that all *Pseudomonas* isolates showed more or less pronounced antifungal activity, whereby the mycelial growth of *Fusarium* species was inhibited in the range of 3.33% (for

Pseudomonas isolates PB4 and PB5 toward *F. tricinctum*) to 77.78% (for *Pseudomonas* isolates K29 and K35 toward *F. oxysporum*).
The highest percentage of growth inhibition was caused by *Pseudomonas* isolates K29 (from 35.71% toward *F. equiseti* to 77.78% toward *F. oxysporum*) and K35 (from 37.50% toward *F. semitectum* to 77.78% toward *F. oxysporum*).
The lowest percentage of inhibition was caused by the following *Pseudomonas* isolates: PB4 (from 3.33% toward *F. tricinctum* to 48.89% toward *F. oxysporum*), PB5 (from 3.33% toward *F. tricinctum* to 51.11% toward *F. oxysporum*), PBA12 (from 13.33% toward *F. tricinctum* to 51.11% toward *F. verticillioides*), PD5 (from 13.33% toward *F. tricinctum* to 53.33% toward *F. verticillioides*).
In general, *F. oxysporum* and *F. verticillioides* showed the highest sensitivity to antibiotic-producing *Pseudomanas* isolates.
Antifungal activity of indigenous *Pseudomonas* isolates was also confirmed in other investigation (Jošić et al., 2012). In addition, *in vitro* assays in previous studies (Velusamy et al., 2011; Shojaii and Abdollahi Fard, 2012) revealed high sensitivity of *F. oxysporum* to *Pseudomonas* sp. as in the present research. As pronounced by other authors (Karimi et al., 2012), PGPR can be used in the biocontrol of phytopathogens.

Table 1. Antifungal activity of selected *Pseudomonas* sp. isolates toward *Fusarium* species (F1 - *Fusarium tricinctum*; F2 - *F. sambucinum*; F3 - *F. equiseti*; F4 - *F. heterosporum*; F5 - *F. sporotrichioides*; F6 - *F. semitectum*; F7 - *F. verticillioides*; F8 - *F. oxysporum*)

Pseudomonas sp. isolates	*Fusarium* species							
	F1	F2	F3	F4	F5	F6	F7	F8
PB4	3.33*	25.71	21.43	20.00	35.56	18.75	42.22	48.89
PB5	3.33	25.71	28.57	8.00	40.00	37.50	42.22	51.11
K38	23.33	17.14	28.57	24.00	33.33	43.75	55.56	44.44
Q34	16.67	17.14	21.43	24.00	33.33	43.75	51.11	51.11
PBA12	13.33	14.29	28.57	8.00	22.22	37.50	51.11	48.89
PD5	13.33	5.71	21.43	12.00	22.22	43.75	53.33	46.67
C7	43.33	34.29	21.43	24.00	44.44	12.50	57.78	51.11
C8	36.67	28.57	21.43	20.00	44.44	25.00	53.33	51.11
Q16P	56.67	42.86	35.71	40.00	66.67	37.50	60.00	71.11
K29	66.67	54.29	35.71	44.00	64.44	43.75	66.67	77.78
K35	60.00	54.29	42.86	40.00	64.44	37.50	66.67	77.78

*Inhibition (in %)

CONCLUSIONS

Biological control of *Fusarium* species, one of the most aggressive isolates from medicinal plants in Serbia, isolated from anise, is an ecological method of plant protection.
Our investigation confirmed more or less pronounced antifungal activity of all tested *Pseudomonas* isolates, whereby the most pronounced activity was observed for K29 and K35 strains. Regarding the *Fusarium* species, the highest sensitivity to antibiotic-producing *Pseudomanas* isolates was observed for *F. oxysporum* and *F. verticillioides*.
Our findings impose that the studied *Pseudomonas* isolates have potential in controlling plant diseases caused by *Fusarium* spp., whereby the bacterial isolates with the highest inhibitory potential will be selected for further experiments.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Project III46007.

REFERENCES

Ayyadurai N., Ravindra Naik P., Sakthivel N., 2007. Functional characterization of antagonistic fluorescent pseudomonads associated with rhizospheric soil of rice (*Oryza sativa* L.). Journal of Microbiology and Biotechnology, 17(6):919-927.

Cattelan A.J., Hartel P.G., Fuhrmann J.J., 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Science Society of America Journal, 63(6):1670-1680.

Couillerot O., Prigent-Combaret C., Caballero-Mellado J., Moënne-Loccoz Y., 2009. *Pseudomonas fluorescens* and closely related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. Letters in Applied Microbiology, 48(5):505-512.

Fernando W.G.D., Nakkeeran S., Zhang Y., 2005. Biosynthesis of antibiotics by PGPR and its relation in biocontrol of plant diseases. In: Siddiqui Z.A. (Ed.), PGPR: Biocontrol and Biofertilization, Springer Science, Dordrecht, The Netherlands, 67-109.

Haas D., Défago G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature Reviews Microbiology, 3(4):307-319.

Heydari A., Pessarakli M., 2010. A review on biological control of fungal plant pathogens using microbial antagonists. Journal of Biological Sciences, 10(4):273-290.

Jošić D., Protolipac K., Starović M., Stojanović S., Pavlović S., Miladinović M., Radović S., 2012. Phenazines producing *Pseudomonas* isolates decrease *Alternaria tenuissima* growth, pathogenicity and disease incidence on cardoon. Archives of Biological Sciences, Belgrade, 64(4):1495-1503.

Karimi K., Amini J., Harighi B., Bahramnejad B., 2012. Evaluation of biocontrol potential of *Pseudomonas* and *Bacillus* spp. against *Fusarium* wilt of chickpea. Australian Journal of Crop Science, 6(4):695-703.

Nair C.B., Anith K.N., 2009. Efficacy of acibenzolar-S-methyl and rhizobacteria for the management of foliar blight disease of amaranth. Journal of Tropical Agriculture, 47(1):43-47.

Nelson P.E., Toussoun T.A., Marasas W.F.O., 1983. *Fusarium* species: an illustrated manual for identification. Pennsylvania State University Press, University Park, Pennsylvania, USA, 193.

Nihorembere V., Ongena M., Smargiass M., 2011. Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnologie, Agronomie, Société et Environnement, 15(2):327-337.

Ogbebor N., Adekunle A.T., 2005. Inhibition of conidial germination and mycelial growth of *Corynespora cassiicola* (Berk and Curt) of rubber (*Hevea brasiliensis* Muell. Arg.) using extracts of some plants. African Jornal of Biotechnology, 4(9):996-1000.

Pierson III L.S., Pierson E.A., 2010. Metabolism and function of phenazines in bacteria: impacts on the behavior of bacteria in the environment and biotechnological processes. Applied Microbiology and Biotechnology, 86(6):1659-1670.

Shojaii A., Abdollahi Fard M., 2012. Review of pharmacological properties and chemical constituents of *Pimpinella anisum*. ISRN Pharmaceutics, 510795.

Showkat S., Murtaza I., Laila O., Ali A., 2012. Biological control of *Fusarium oxysporum* and *Aspergillus* sp. by *Pseudomonas fluorescens* isolated from wheat rhizosphere soil of Kashmir. IOSR Journal of Pharmacy and Biological Sciences, 1(4):24-32.

Velusamy P., Ko H.S., Kim K.Y., 2011. Determination of antifungal activity of *Pseudomonas* sp. A3 against *Fusarium oxysporum* by high performance liquid chromatography (HPLC). Agriculture, Food and Analytical Bacteriology, 1:15-23.

Zehnder G.W., Murphy J.F., Sikora E.J., Kloepper J.W., 2001. Application of rhizobacteria for induced resistance. European Journal of Plant Pathoogy, 107(1):39-50.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRELIMINARY DATA ON PESTS OCCURRENCE ON SAFFLOWER CROP UNDER GREENHOUSE CONDITIONS

Aurora DOBRIN[1], Roxana CICEOI[1], Vlad Ioan POPA[1], Ionela DOBRIN[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Laboratory of Diagnosis and Plant Protection of Research Center for Studies of Food Quality and Agricultural Products, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: roxana.ciceoi@gmail.com

Abstract

Safflower is a very important oilseed crop with multiple uses in food, pharmaceutic, cosmetic, varnish and paint industry. The quality of safflower flowers and seed yield rely on successful and integrated pest management solution. The safflower crop was tested in Romania in the last decades and the results show a high adaptability of this species to our pedoclimatic conditions, which led to a yield higher than 2000kg/ha, for the studied varieties. Our observations were carried out in the Research Greenhouse of University of Agronomic Science and Veterinary Medicine from Bucharest, in 2016, on Carthamus tinctorius L., which represent the first attempt in growing safflower in greenhouse conditions in our country. The most damaging pests that were identified were Tetranychus urticae Koch. and Trialeurodes vaporariorum Westood, two threatening polyphagous pest all around the world, causing serious yield losses, especially in greenhouses. Their presence was associated with the high temperature in June and July. Besides the introductory review of the most important safflower pest in the world, this study gives new and important insights about the safflower crop response to associated greenhouse pests and allowed a closer analyze using the electronic microscopy of the white fly eggs and eggs hatching characteristics. Our observation on safflower might be a premise for new control strategies against the white fly.

***Key words**: Carthamus tinctorius, Tetranychus urticae, Trialeurodes vaporariorum, mature eggs, eggs hatching.*

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a very important multipurpose crop, that is used in producing herbal drugs, cosmetics, natural food coloring, high oleic and high linoleic oil, natural dye, oil for painting and animal feed.
Safflower is a drought and salt-resistant oilseed crop. Safflower grows on various types of soils, but the highest yield may obtain on clay, sandy lands with neutral pH, well drained in depth. Safflower crops on lands with excessive humidity have a high risk of contacting specific diseases and pests (Kizil et al., 2008; Amini, 2014; Hussain et al., 2016).
The most important pest insects feeding inside the flower heads of safflower mentioned until the present moment are *Acanthiophilus helianthi*, *Chaetorellia carthami*, *Trellia luteola*, *Larinus flavescens*, *Larinus liliputanus and Helicoverpa peltiger* (Saeidi and Nur Azura, 2011).
The safflower fly, *Acanthiophilus helianthi* Rossi (*Diptera*: *Tephritidae*) is one of the most important pests of safflower allover the world. Larval feeding can causes important losses, disrupt plant metabolism with a negative influence on number of flower buds and decrease quality and quantity of the crop yield (Riaz and Sarwar, 2013; Saeidi et al., 2013). In Iran both safflower fly and Silver Y moth cause major damage to the safflower crops. (Saeidi et al., 2011; Esfahani et al., 2012).
Safflower anthodium can be infested with the tephritid fruit flies as *Acanthiophilus helianthi* Rossi and *Chaetorellia carthami* Stackelberg, but there are five associated hymenopteran parasitoid species, namely *Bracon luteator* Spinola; *B. intercessor* Nees (*Braconidae*); *Eurytoma varicolor* Silvestri; *E. rtellii* Domenichini (*Eurytomidae*) and *Torymus rubi* (Schrank) (*Torymidae*) that are keeping the tephiritid populations under control (Basheer et al., 2014).

In Iraq, Israel, and Kirgizstan, the species from the genus *Chaetorellia* (*Diptera*: *Tephritidae*), especially the *C. carthami* Stackelberg was reported as a safflower pest. (Saeidi et al., 2015).

The most important pest insects feeding outside the safflower anthodium are *Oxycarenus pallens*, *Oxycarenus hyalipennis, Lygus* sp. (Saeidi and Nur Azura, 2011; Esfahani et al., 2012).

The most important pest insects feeding on the whole safflower plant are *Uroleucon compositae*, *Pleotrichophorus glandolosus*, *Brachycaudus helichrysi*, *Neoaliturus fenestratus*, *Euscelis alsius*, *Macrosteles laevis*, *Psammotettix striatus*, *Circulifer haematoceps*, *Thrips tabaci*, *Aeolothrips collaris*, *Haplothrips sp*, *Helicoverpa peltigera* (Saeidi and Nur Azura, 2011; Esfahani et al., 2012). The safflower aphid (*Uroleucon compositae* Theobald) is the major safflower pest in India because in high infestations can damage the crop completely. The yield losses of safflower due to aphids are reported to be 24,2 - 72%. (Esfahani et al., 2012; Singh and Nimbkar, 2016). Among the 36 species of pests damaging safflower in India, the safflower aphid, the capsule borer, *Helicoverpa armigera* (Hubner) and leaf eating caterpillar, *Perigea capensis* (Walker) are considered to be the most important pests of the crop (Esfahani et al., 2012; Saeidi et al., 2015).

In the Mediterranean region there were reports about *Acanthiophilus helianthi*, *Heliothis peltigera* SchiV. (*Noctuidae*), *Chaetorellia carthami* Stackelberg, *Ch. jaceae* R.D., *Terellia luteola* Wiedemann, *Urophora mauritanica* Macquart (*Tephritidae*), *Larinus grisescens* Gyll., *Larinus syriacus* Gyll., *Larinus orientalis* Cap., and *Larinus ovaliformis* Cap. (*Curculionidae*) on the Xower heads; and *Lixus speciosus* Mill. (*Curculionidae*), Agapanthia sp. (*Cerambycidae*), four *Chloridea* spp., *Plusia gamma* L. (*Noctuidae*), *Pyrameis cardui* L. (*Nymphalidae*), and *Cassida palaestina* Reiche (*Chrysomelidae*) damaging the safflower (Smith et al., 2006).

In central and northern Greece, *Botanophila turcica* (*Diptera*: *Anthomyiidae*) was reported recently for the first time on safflower. The larvae of this fly tunnel through the rosette meristem and root of the developing host plant, causing deformation of the developing leaves and occasionally plant losses. *B. turcica* has been reported to attack only rosettes of the invasive saffron thistle *Carthamus lanatus* L. and has, therefore, been suggested as a potential biological control agent of *C. lanatus* (Tsialtas et al., 2013).

Other reported pests with low impact are scarab beetle *Epicometis hirta*), Egyptian cotton leaf, *Spodoptera littoralis*), wireworms, *Limonius* spp., cotton boll worm, *Heliothi obsoleta Lasioderma serricorne*, *Stegobium penliceum and Trogodema* (Esfahani et al., 2012).

Another important pest category is represented by mites. They cause damage by sucking cell contents from leaves. At first, the damage shows up as a stippling of light dots on the leaves; sometimes the leaves take on a grey, yellow or bronze colour. Necrotic spots occur in the advanced stages of leaf damage. Spider mites are highly polyphagous pests (Godfrey, 2011; Fasulo and Denmark, 2016). *Tetranychus urticae* Koch (*Acari*, *Tetranychidae*) is an notorious mite species causing serious yield losses almost all over the world. It is considered to be a temperate zone species, but it is also found in the subtropical regions (Esfahani et al., 2012; Fasulo and Denmark, 2016; Jiao et al., 2016; Rector et al., 2016).

For an integrated pest management a very important key are the natural enemies that limit pests. In Egypt, the safflower capsule fly is attacked by three species of parasitoid wasps from the families of *Eulophidae* (*Pronatalia* sp.), *Torymidae* (*Antistrophophlex conthurnatus*) and *Pteromalidae* (*Pteromalus* sp.) (Saeidi et al., 2015).

The source of tolerance to aphids can be present in the locally available germplasm. Aphid resistance in safflower is reported to be under the control of both additive and nonadditive gene actions with a predominance demonstrated for nonadditive gene action (Singh and Nimbkar, 1993). Breeding for aphid resistance has been initiated recently in India since it is the most economical, time-tested, and eco-friendly method for controlling aphids. Aphid tolerant safflower keeps the environment safe by way of avoiding chemical usage (Esfahani et al., 2012; Singh and Nimbkar, 2016).

Important genera include the predatory mites, *Amblyseius*, *Metaseiulus*, and *Phytoseiulus*; the lady beetles, *Stethorus picipes*; the minute pirate bugs, *Orius*; *Scolothrips sexmaculatus, Leptothrips*; and the lacewing larvae, *Chrysopa*. *Galendromus occidentalis*. In greenhouses, the ghost ant, *Tapinoma melanocephalum* (Fabricius), a pest in itself, was also reported as a significant predator (Godfrey, 2011; Fasulo and Denmark, 2016).
Other pests control strategies could be: a very good sanitation, that is a key for controlling pests in greenhouses, weed control, clean up all debris from previous crops, temporary quarantine and inspection of all plants upon arrival from other greenhouse, and regular monitoring of stock plants used for propagation, seed selection and proper seed rate, respecting proper sowing time, varietal selection and crop rotation; insect grow regulators are a least-toxic pesticide control option for pests, bio rational pesticides and fertilizers levels (Greer and Diver, 1999; Gupta and Gupta, 2016).
The aim of this work is to present the associate arthropod pests identified on safflower crop grown in greenhouse in 2016.

MATERIALS AND METHODS

The observations were made in the Research Greenhouse of University of Agronomic Sciences and Veterinary Medicine from Bucharest on *Carthamus tinctorius* L. crop in greenhouse. For our country, it is a novelty to obtain safflower crop in the greenhouse. The sowing was done in 19 April 2016 and the harvest on 12 July 2016. During the growing period, we observed several pests affecting the leaves and anthodia. Both the pests and the infested leaves were collected in entomological jars once a week and after each inspection, the pests were immediately analysed at the stereomicroscope. After drying at room temperature, the samples with whitefly eggs exuvia were kept in laboratory, in plastic Petri dishes. The safflower infected leaves and anthodia were analysed with a Leica S8 APO stereomicroscope and with the Scanning Electron Microscope SEM FEI Inspect S50. For both observation methods, there is no sample preparation needed.

RESULTS AND DISCUSSIONS

Trialeurodes vaporariorum eggs analyse

The first greenhouse whitefly eggs were noticed immediately after the leaf emergence. Heavy infestations, between 27 and 48 eggs/cm^{-1} have been observed on 10 May, at 20 days after sowing (figure 1), in the 8 real leaves phenological growing stage.

Figure 1. Heavily infested safflower leaves with greenhouse whitefly eggs

The leaves were also very soon infested by *Tetranychus urticae* Koch (figure 2), the silk webbing on infested leaves being easily detectable and the spherical and translucent eggs being visible at the stereomicroscope. A density of 2 to 5 adult mites and 6 to 12 mite eggs on cm^{-1} has been estimated at the same phenological growing stage.

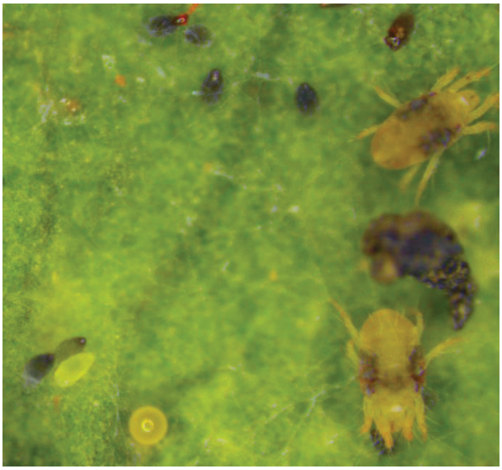


Figure 2. *Tetranychus urticae* eggs, adults and greenhouse whitefly eggs

In the literature it is often cited that the eggs of greenhouse whitefly are pale yellow when first laid and turn to darker colour, until black before hatching (figure 3).

We found no data about the whitefly eggshell neither about its description or its opening structures. This fact is usually undetected, as the leaves are usually covered by sooty mould.

The humidity and temperature conditions correlated with the morpho-anatomical safflower cuticle allowed *T. vaporariorum* eggs to remain on the lamina after the eggs hatching, so that we could observe and analysed the eggs shells opening with the Scanning Electron Microscope (figures 4 and 5).

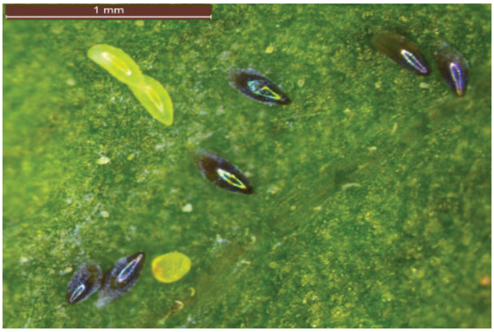


Figure 3. Newly laid greenhouse whitefly eggs (translucent colour) and more mature ones (dark colour)

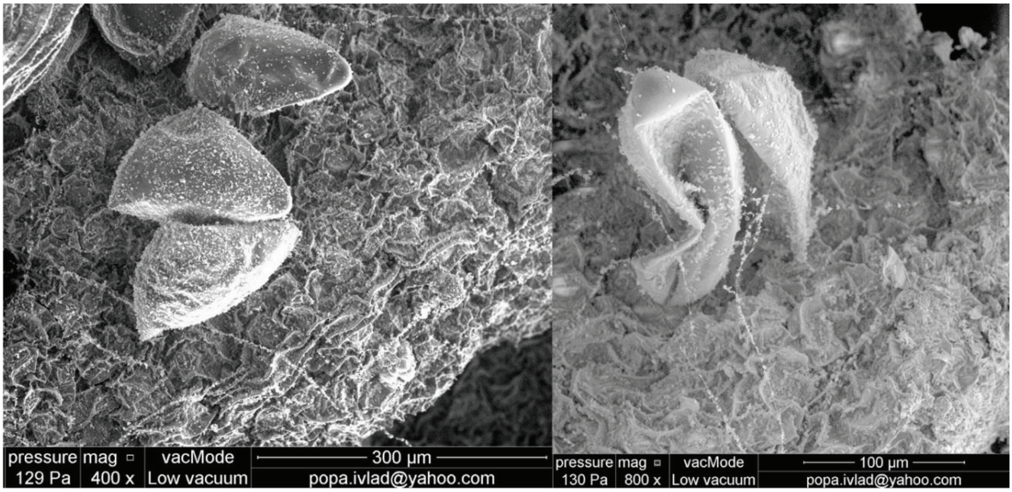


Figure 4. Greenhouse whitefly eggs and details of the safflower lower cuticle

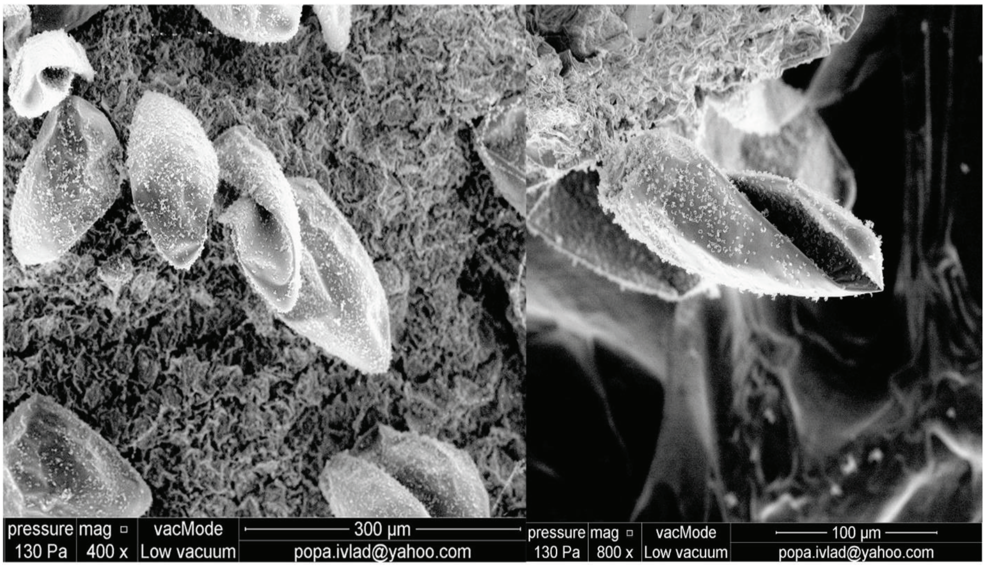


Figure 5. The opening pattern of greenhouse whitefly eggs during hatching

The observed longitudinal opening of the egg shell could offer new insights for the integrated control measures against the greenhouse whitefly. On our knowledge, this aspect hasn't been discussed so far, so further studies are needed.

CONCLUSIONS

The humidity and temperature conditions proved to be an important factor, high temperatures facilitating the observation of new morphological aspects. In the same time, the morpho-anatomical safflower cuticle surface proved to be a perfect medium in preserving the whitefly egg shells.

This new information about the pests associated with *Carthamus tinctorius* L. crop are very useful in the context of food and nutritional safety and sustainable production of safflower in our country and new strategies are required to raise safflower productivity sustainably.

REFERENCES

Amini H., Arzani A., Karami M., 2014. Effect of water deficiency on seed quality and physiological traits of different safflower genotypes. Turkish Journal of Biology Vol.38, Issue: 2, 271-282, DOI: 10.3906/biy-1308-22.

Basheer A., Asslan L., Abdalrazaq F., 2014. Survey of the Parasitoids of the Tephritid Fruit Flies of the Safflower *Carthamus tinctorius* (*Asteracea*) in Damascus, Syria. Egyptian Journal of Biological Pest Control, Vol.24, 169-172.

Esfahani M.N., Alizadeh G., Zarei Z., Esfahani M.N., 2012. The Main Insect Pests of Safflower on Various Plant Parts in Iran. Journal of Agricultural Science and Technology A 2, 1281-1289, ISSN 1939-1250.

Fasulo T.R., Denmark H. A., 2016. Twospotted Spider Mite. *Tetranychus urticae* Koch (*Arachnida: Acari: Tetranychidae*). EENY150 , DPI Entomology Circular 89, Entomology and Nematology Department, UF/ IFAS Extension, 1-5.

Godfrey L. D., 2011. Spider Mites. PEST NOTES, Statewide Integrated Pest Management Program, Publication 7405, UC Statewide Integrated Pest Management Program, University of California, Davis, CA 95616, available online at: http://ipm.ucanr.edu/PMG/PESTNOTES/pn7405.html.

Greer L., Diver S., 1999. Integrated pest management for greenhouse crops.Pest management systems guide ATTRA 8 0 0 -3 4 6 -9 1 4 0, 1-34.

Gupta R.D., Gupta S.K., 2016. Chapter 1 – Strategies for Increasing the Production of Oilseed on a Sustainable Basis. Breeding Oilseed Crops for Sustainable Production. Opportunities and Constraints. 1–18, http://dx.doi.org/10.1016/B978-0-12-801309-0.00001-X.

Hussain M.I., Lyra D.A, Farooq M., Nikoloudakis N., Khalid N., 2016. Salt and drought stresses in Safflower: a review. Agronomy for Sustainable Development Vol. 36, Issue: 1, DOI: 10.1007/s13593-015-0344-8.

Jiao R., Xu C.X., Yu L.C., He X.Z., Qiao G.Y., He L.M., Li L.T., 2016. Prolonged coldness on eggs reduces immature survival and reproductive fitness in *Tetranychus urticae* (*Acari*: *Tetranychidae*). Systematic and Applied Acarology, 21(12), 1651-1661. http://doi.org/10.11158/saa.21.12.6; ISSN 1362-1971 (print), ISSN 2056-6069 (online).

Kizil S., Cakmak O., Kirici S., Inan M., 2008. A comprehensive study on safflower (*Carthamus tınctorius* L.) in semi-arid conditions. Biotechnol Biotec Eq. 22: 947- 953.

Rector B.G., Czarnoleski M., Skoracka A., Lembicz M., 2016. Change in abundance of three phytophagous mite species (*Acari*: *Eriophyidae*, *Tetranychidae*) on quackgrass in the presence of choke disease. Exp Appl Acarol.; 70: 35–43. doi: 10.1007/s10493-016-0060-3.

Riaz M., Sarwar M., 2013. A New Record of Safflower Fly *Acanthiophilus helianthi* (Rossi) of Genus *Acanthiophilus* Becker in Subfamily *Tephritinae* (*Diptera*: *Tephritidae*) from the Fauna of Pakistan. Research & Reviews: Journal of Agriculture and Allied Sciences, ISSN: E 2347-226X, P 2319-9857, 39-44.

Saeidi K.,Nur Azura A., 2011. A survey on pest insect fauna of safflower fields in the Iranian Province of Kohgiloyeh and Boyerahmad. African Journal of Agricultural Research Vol. 6(19), pp. 4441-4446, DOI: 10.5897/AJAR11.966, ISSN 1991-637X.

Saeidi K., Nur Azura A., Omar D., Abood F., 2011. Pests of safflower (*Carthamus tinctorious* L.) and their natural enemies in Gachsara, Iran. South Asian Journal of Experimental Biology, Vol 1, No 6 (2011) ISSN: 2230-9799, 286-291.

Saeidi K., Nur Azura A., Omar D., Abood F., 2013. Population Dynamic of the Safflower Fly, *Acanthiophilus Helianthi* Rossi (*Diptera: Tephritidae*) in Gachsaran Region, Iran. Entomol Ornithol Herpetol 2013, 2:1 Volume 1, Issue 1, ISSN: 2161-0983 http://dx.doi.org/10.4172/2161-0983.1000103.

Saeidi K., Mirfakhraei S., Mehrkhou F., Valizadegan O., 2015. Biodiversity of Insects Associated with Safflower (*Carthamus tinctorius*) Crop in Gachsaran. Iran. Journal of Entomological and Acarological Research, Vol 47, No 1, 26-30, DOI: 10.4081/jear.2015.1910.

Singh V., Nimbkar N., 2016. Chapter 7–Safflower. Breeding Oilseed Crops for Sustainable Opportunities and Constraints Production, 149-167, ISBN: 978-0-12-801309-0, http://dx.doi.org/10.1016/B978-0-12-801309-0.00007-0.

Smith L., Hayat R., Cristofaro M., Tronci C., Tozlu G., Lecce F., 2006. Assessment of risk of attack to saffower by *Ceratapion basicorne* (*Coleoptera*: *Apionidae*), a prospective biological control agent of *Centaurea solstitialis* (*Asteraceae*). Biological Control 36, 337–344.

Tsialtas I.T., Michelsen V., Koveos D.S., 2013. First report of *Botanophila turcica* (*Diptera*: *Anthomyiidae*) on safflower *Carthamus tinctorius* L. in Greece. Journal of Biological Research-Thessaloniki, Vol 19, 80-82 ISSN : 1790-045X.

DP 03: Morphological Identification of Spider Mites (*Tetranychidae*) Affecting Imported Fruits, 2014 available online at: https://www.nappo.org/files/3714/3782/0943/DP_03_Tetranychidae-e.pdf.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ACHIEVEMENT OF SOME FUNCTIONAL INGREDIENTS FROM TOMATO WASTE AND WINEMAKING BY-PRODUCTS

Monica CATANĂ[1], Luminița CATANĂ[1], Enuța IORGA[1], Adrian Constantin ASĂNICĂ[2], Anda-Grațiela LAZĂR[1], Monica-Alexandra LAZĂR[1], Nastasia BELC[1]

[1]National Research & Development Institute for Food Bioresources, IBA Bucharest, 6 Dinu Vintila, District 2, 021102 Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: mcatana1965@gmail.com

Abstract

A major problem facing the food industry is accumulation, handling and disposal of waste from the processing of raw materials. Therefore, valorisation of such waste by achievement of functional ingredients, leading to increasing of nutritional quality and antioxidant potential of foods is of real interest. Among waste and by-products from the processing industry of vegetables and fruits with valorisation potential are tomato waste, dark colour grape seed and skin (red, purple, and black). In this paper are presented results of the performed research for achievement of some functional ingredients (flours) from tomato waste and winemaking by-products (grape pomace and grape seed). Tomato waste and winemaking by-products were subjected to convective drying process at temperature of 50°C, in order to protect bioactive compounds (vitamins, phenolic compounds etc.), to a moisture which allows their milling and conversion into flours and, at the same time, their stability in terms of quality. The achieved functional ingredients were evaluated from sensory, physic-chemical and microbiological point of view. Flour obtained from tomato waste is characterized by content in carotenoids (lycopene – 225.92 mg/kg; ß-carotene - 16.22 mg/kg), protein (17.62%), minerals, total fibre (59.47%), total polyphenol (18.76 mg GAE/g). Flours achieved from winemaking by-products are characterized by content of protein (10.53-14.63%), minerals, total fibre (58.06-66.06%) and total polyphenol (200.15-322.75 mg GAE/g). Antioxidant capacity of flour achieved from tomato waste was 1.62 mg Trolox Equivalents/g, and of flour achieved from winemaking by-products varied in the range (40.75–51.25 mg Trolox Equivalents/g). Microbiological analysis showed that flours obtained from tomato waste and winemaking by-products (grape pomace and grape seed) are under the provisions of the legislation in force.

Key words: *tomato, waste, grape, pomace.*

INTRODUCTION

Numerous studies have shown the presence of bioactive compounds in various types of agro-industrial waste, with potential application in the industry.

Their reuse would reduce environmental risks caused by disposal, besides providing a source of profitability for populations living around industrial regions (Anastasiadi et al., 2008).

Tomatoes (*Lycopersicon esculentum* L.) are cultivated worldwide for their fruits, registering an annual production of 161.8 million tonnes (FAOSTAT, 2012). Regular consumption of tomatoes and processed tomato products was correlated with a reduction in susceptibility to various cancers and cardiovascular diseases (Borguini and Da Silva Torres, 2009). These positive effects are due to antioxidant compounds present in tomatoes, such as vitamins C and E, carotenoids, polyphenols, which play a key role in the mechanism of health protection by neutralizing free radicals (Ray et al., 2011). Also, tomatoes are an important source of trace elements, namely, selenium, copper, manganese and zinc, which are cofactors of antioxidant enzymes (Martinez-Valverde et al., 2002). Due to the complex biochemical composition, consumption of fresh or processed tomatoes (juice, piuree, paste, ketchup etc.) has beneficial effects on the human body: cardioprotective (Palomo et al., 2009), anti-platelet (platelet aggregation inhibition) (Fuentes et al., 2012), decreasing of triglycerides and cholesterol level in the blood (Hsu et al., 2008), reducing of oxidative stress induced by postprandial lypemia (increase of lipid level in the blood after lunch) (Burton-Freeman et al., 2012).

Studies concerning localization of antioxidant compounds in the different fractions of tomatoes (epicarp, seed and pulp) confirmed that, in all tomato cultivars under study, within the epicarp are the highest concentrations of phenolic compounds, flavonoids, lycopene and ascorbic acid. At the same time, tomato epicarp has an antioxidant activity higher than the pulp and seed fractions (George et al., 2004; Toor and Savage, 2005). Also, several studies showed that tomato seed are rich in nutrients including: carotenoids, proteins, polyphenols, minerals, fibres and oils (Liadakis et al., 1995; Persia et al., 2003; Toor and Savage, 2005; Demirbaş, 2010; Eller et al., 2010; Zuorro et al., 2013).

Million tonnes of tomatoes are annualy processed to juice, sauces, piurees, paste and tomato canned, generating high amounts of tomato peel, pulp and seed, which are industrial waste (Papaioannou and Karabelas, 2012). When tomatoes are processed and converted into ketchup, sauces or juice, waste is generated representing 3-7% of the tomato mass introduced in the manufacture process (Savatović et al., 2010).

Tomato seed contains about 24.5% crude protein and have the highest content of glutamic acid and aspartic acid (Persia et al., 2003). Del Valle et al. (2006) evaluated the chemical composition of the waste resulted from the industrial processing of tomatoes to paste, in various stages of the process flow (after pulper, after finisher, before turbopress and after turbopress). Average composition (in dry weight basis) of tomato pomace was the following: 59.03% fibres, 25.73% total sugars, 19.27% protein, 7.55% pectins, 5.85% total fat and 3.92% minerals.

Aghajanzadeh et al. (2010) have shown that the dried tomato waste contains 22.6–24.7% protein, 14.5–15.7% fat and 20.8–23.5% fibres and, at the same time, represents a source of vitamins B_1, B_2 and A. In addition, tomato waste contains essential aminoacids, and tomato seed contains high concentrations of minerals (Fe, Mn, Zn and Cu). Tomato peel contains significantly higher concentrations of lycopene and β-carotene compared to the pulp and seed (Papaioannou and Karabelas, 2012). Majority of flavonoids is found in the peel of tomatoes.

Vitis vinifera L. production is widespread throughout the world, exceeding 68 million tonnes (FAOSTAT, 2010). As grape seeds comprise about 5% of the fruit weight (Choi and Lee, 2009), more than 3 million tonnes of grape seeds are discarded annually world-wide (Fernandes et al., 2012). Grape seeds represent a significant part in pomace, namely 38–52% of dry matter (Maier et al., 2009). Grape pomace represents a mixture of grape peel, seed and trace of pulp, resulted after wine obtaining. Grape seed and skin constituents have been shown to have health-functional activities as LDL cholesterol-lowering functional foods (Chen et al., 2011).

Composition of grape seeds is represented by 40% fibres, 16% essential oil, 11% protein, 7% complex phenolic compounds like tannins, and also sugars and minerals (Campos et al., 2008). Ca, K, Mg, Na and P are the most important minerals in grape seed (Ozcan, 2010). White grape seeds have a content of total polyphenols (on average 58.23±3.978 g/kg DM) higher compared to black grape seed (32.22±2.197 g/kg DM). Also, in grape seed, γ-tocotrienol is the most abundant (46.31±13.37 mg/kg DM), followed by α-tocotrienol (20.00±7.81 mg/kg DM) and α-tocopherol (12.45±4.85 mg/kgDM) (Lachman et al., 2013).

Grape pomace and its derivatives have use potential in diabetes management (Hogan et al., 2010). Three polyphenolic compounds in grape seed (gallic acid, catechin and epicatechin) inhibited pancreatic cholesterol esterase and may increase control on bioavailability of dietary cholesterol and cholesterol ester derivative, thus limiting the absorption of free cholesterol in blood (Ngamukote et al., 2011).

In this paper are presented results of the performed research for achievement of some functional ingredients (flours) from tomato waste and winemaking by-products (grape pomace and grape seed).

MATERIALS AND METHODS

Samples

Tomato waste resulted from tomato processing to juice within the Pilot Experiments Plant for Fruits and Vegetables Processing in IBA

Bucharest. Winemaking by-products (black grape seed and pomace) were collected after producing wine in the households in rural areas. Till processing, winemaking by-products were shipped and stored under refrigeration (3 °C). Tomato waste and winemaking by-products were subjected to dehydration process in a convection dryer at temperature 50 ºC to a moisture which allows their milling and conversion into flours and, at the same time, their stability in terms of quality. Milling of dried semi-finished products was performed by using Retsch mill. The achieved functional ingredients (flours) were packed in glass containers, hermetically sealed, protected by aluminum foil against light and stored in dry and cool areas (temperature of maximum 20 ºC), till to the sensory, physic-chemical and microbiological analysis). In Figure 1 are presented the flours mentioned above.

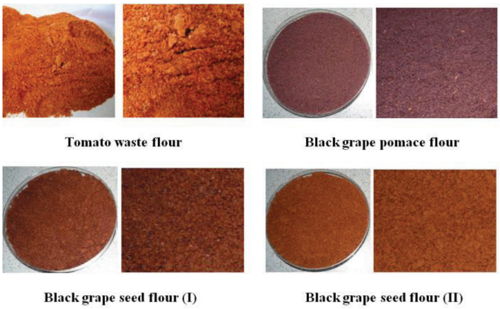


Figure 1. Flours achieved from tomato waste and winemaking by-products

Methods

Sensory analysis

Sensory analysis (appearance, taste and smell) was performed by descriptive method.

Physic-chemical analysis

Measurement of the color parameters of samples was performed at room temperature, using a HunterLab colorimeter, equipped with Universal Software V4.01 Miniscan XE Plus programme, to register CIELab parameters (the Commission Internationale de l'Eclairage - CIE), L^*, a^* and b^*: L^* - color luminance (0 = black, 100 = white); a^* - red-green coordinate (-a = green, +a = red); b^* - yellow-blue coordinate (-b = blue, +b = yellow).

Moisture determination was performed with Ohaus Moisture Analyzer MB45 at temperature 105 °C.

Protein content was determined by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 979.09, 2005). Fat content was determined according to AOAC Method 963.15, and ash content according to AOAC Method 923.03 (AOAC, 2005).

In order to determine minerals samples were mineralized by calcination, with the addition of hydrochloric acid and hydrogen peroxide. The minerals sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and zinc (Zn) were determined by atomic absorption spectrophotometer (type *AAnalyst* 400, Perkin–Elmer). The minerals iron (Fe) and selenium (Se) were determined by Graphite Furnace Atomic Absorption Spectrophotometer (type *AAnalyst* 600, Perkin–Elmer).

Total dietary fibre (TDF) was determined by enzymatic method using the assay kits: K-TDFR ''Total dietary fibre'' (AOAC Method 991.43).

Lycopene and beta carotene content were performed by using spectrophotometric method developed by Nagata and Yamashita (1992).

Total polyphenol content

Total polyphenol content was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The extraction of phenolic compounds was performed in methanol:water 50:50, and the absorbance of the extracts was determined at a wavelenght λ = 755 nm. Results were expressed as mg of gallic acid equivalents (GAE) per g flour (black grape seed flour, black grape pomace flour, tomato waste flour).

Antioxidant capacity

The DPPH scavenging radical assay was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f (Trolox concentration), in the concentration range 0-0.4375 mmol/L and the results were expressed as mg Trolox Equivalents per g flour (black grape seed flour, black grape pomace flour, tomato waste flour).

Microbiological analysis

The water activity (*A*w) was determined by an instrument Aquaspector AQS-2-TC, Nagy. The measurements were performed at 25ºC. Yeast and mold were determined by the method SR ISO 21527-1:2009. *Enterobacteriaceae* was determined according to the SR ISO 21528-2:2008 method and *Escherichia coli* by SR ISO 16649-2:2007 method. *Salmonella* was determined by the method SR EN ISO 6579:2003/AC:2006.

RESULTS AND DISCUSSIONS

Sensory analysis

After sensory analysis it was found that the obtained flours have specific characteristics. Tomato waste flour is in the form of orange powder with pleasant taste and smell, characteristic. Black grape seed flour and black grape pomace flour are in the form of a dark brown powder, purple tinge, respectively, with specific, pleasant taste and smell.

After the instrumental analysis of color (Figure 2) it was found that black grape seed flour (I) has the darkest color, registering the minimum value of luminance (L* = 24.38), while the tomato waste flour has the lightest color (L* = 59.13).

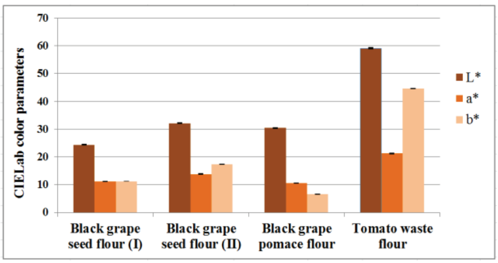


Figure 2. Color parameters of the flours achieved from tomato waste and winemaking by-products

Also, the maximum positive values of parameter a* (red coordinate) and of parameter b* (yellow coordinate) were registered for tomato waste flour.

Physic-chemical analysis

Composition of the flours achieved from tomato waste and winemaking by-products is presented in Table 1.

Water content of grape pomace flour is higher than that reported by Sousa et al., 2014 (3.33±0.04% dry basis), and of grape seed flour is lower than that reported by Aghamirzaei et al., 2015 (7.48±0.73% dry basis).

Table 1. Physic-chemical composition of flours achieved from tomato waste and winemaking by-products

Flour type	Water (%)	Ash (%)	Protein (%)	Fat (%)	Total fibre (%)
Black grape seed flour (I)	4.90±0.12	2.80±0.04	10.53±0.09	15.36±0.17	64.67±1.20
Black grape seed flour (II)	3.91±0.08	2.90±0.04	10.85±0.09	13.17±0.15	66.06±1.22
Black grape pomace flour	4.59±0.10	6.61±0.09	10.63±0.09	8.49±0.10	58.86±1.09
Tomato waste flour	7.64±0.17	4.05±0.05	17.62±0.16	10.38±0.12	59.47±1.10

Water content of tomato waste flour is higher than that reported by Majzoobi et al. (2011) (4.71% dry basis). After physic-chemical analysis it was found that the achieved flours are distinguished by content of total ash, protein and total fibre. Their ash content varied in the range 2.80-6.61% (minimum value was registered for the black grape seed flour (I), and the maximum one for the black grape pomace flour). Ash content of grape seed flour is comparable with that reported by Aghamirzaei et al. (2015) (2.45±0.18% dry basis), and ash content of grape pomace flour is higher compared to that obtained by Sousa et al. (2014) (4.65±0.05% dry basis). Ash content of tomato waste flour is comparable to that reported by Majzoobi et al. (2011) (4.53%).

Flours obtained from winemaking by-products registered close values for protein content (10.53-10.85%), lower than those obtained by Valiente et al. (1995), Llobera & Cañellas (2007) and Bravo & Saura-Calixto (1998) in grape residues (11g/100g, 12g/100g, and 14g/100g). Protein content (17.62%) of tomato waste flour obtained in this study is with 10.65% lower than that reported by Majzoobi et al., 2011 (19.72%). Grape seed flour obtained within the experiments has fat content higher than grape pomace flour. It is noted that grape pomace flour achieved in this experimental study has a fat content significantly higher than that reported by Bampi et al. (2010) in flour grape residues (2.56 g/100g). Grape fats are concentrated, mainly, in seeds and consist in approximately 90% mono-unsaturated fatty acids, known for their beneficial properties, notably for cardiovascular system (Rockenbach et al., 2010). Tomato waste flour had a fat content 2.09 times higher than that found by Majzoobi et al. (2011) in case of powder obtained from tomato waste (4.96%).

Flours obtained from tomato waste and winemaking by-products presented a high total fibre content (58.86-66.06%), the maximum value being registered for grape seed flour (II). Values of this chemical parameter for flours obtained from winemaking by-products are higher than those reported by Aghamirzaei et al. (2015) for grape seed powder (42.74±0.6% dry basis) and Sousa et al. (2014) for grape pomace flour (46.17±0.80% dry basis). Sousa et al. (2014) mentioned that grape pomace flour is a good source of dietary fibre providing 79% insoluble fibre and 21% soluble fibre. Pérez-Jiménez et al. (2008) mentioned that dietary fibre of grapes significantly reduced lipid profile and blood pressure, and these effects were significantly higher than those produced by other dietary fibre, such as oat or psyllium fibre, probably, due to the combined effect of dietary fibre and antioxidants.

Total fibre content of tomato waste flour is lower by 18.19% than that reported by Majzoobi et al. (2011) for tomato pomace powder (72.68%).

Flours obtained from winemaking by-products and tomato waste are an important source of minerals (K, Ca, Mg, Fe, Zn și Se). Their content in minerals is presented in Figures 3 and 4. Flours achieved from winemaking by-products have a high potassium content in the range 1102.35-3406.67 mg/100g, the maxium value being recorded by black grape pomace flour. Their potassium content is higher than that reported by Gül et al. (2013) (2343.10 mg/100 g for whole flour of Öküzgözü pomace, 1587.10 mg/100g for whole flour of Narince pomace, 312.89 mg/100 g for seed flour of Öküzgözü pomace and 458.24 mg/100 g, respectively, for seed flour of Narince pomace).

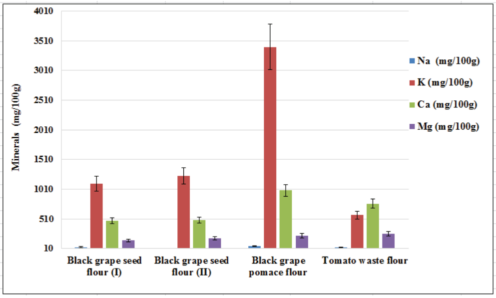


Figure 3. Mineral content (Na, K, Ca, Mg) of the flours achieved from tomato waste and winemaking by-products

Potassium content of tomato waste flour (573.09±64.76 mg/100g) is higher than that reported by Nour et al. (2015) for tomato waste (moisture content = 69.98±0.18%, K = 303.02 mg/100 g). These flours have potassium content higher than that in sodium. The results are in conformity with those obtained by Sousa et al. (2014) which states that this may lead to a balance of minerals, which favours hypertension control.

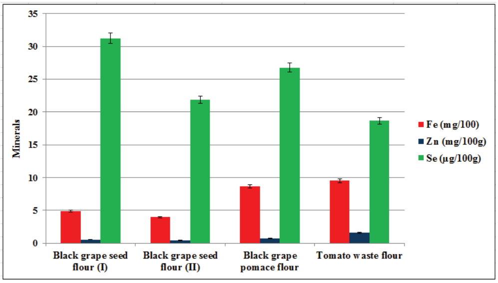


Figure 4. Mineral content (Fe, Zn, and Se) of the flours achieved from tomato waste and winemaking by-products

Calcium and magnesium content of tomato waste flour, black grape seed flour and black grape pomace flour, respectively, are higher than those reported by the other authors (Gül et al., 2013; Sousa et al., 2014).

Iron content of vegetable flours achieved in this study varied in the range 3.97-9.54 mg/100 g. Minimum value was registered for black grape seed flour (II), and the maximum one was registered for tomato waste flour. Grape pomace flour has an iron content of about 2.00 times higher than black grape seed flour. Grape pomace flour achieved in this study has an iron content of about 1.91 times higher than that reported by Lachman et al. (2013) (4.54 mg/100 g), but of about 2.6 times lower than that reported by Gül et al. (2013) (22.52 mg/100 g for whole flour of Öküzgözü pomace and 13.92 mg/100 g, respectively, for whole flour of Narince pomace). At the same time, flours achieved from blake grape seed within this study presented an iron content comparable to that reported by Gül et al. (2013) (2.86 mg/100 g for seed flour of Öküzgözü pomace and 5.13 mg/100 g, respectively, for seed flour of Narince pomace). Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport (Abbaspour et al., 2014). Flours achieved from winemaking by-products registered a low Zn content (0.42 mg/100 g-0.75 mg/100 g), compared to those presented by other authors (Sousa et al., 2014, 0.98±0.702 mg/100 g in case of grape pomace flour and, respectively Lachman et al., 2013, 1.1 mg/100 g in case of grape seed). Tomato waste flour recorded the highest Zn content (1.56 mg/100 g). Zn is an important element of the immune system. Also, Bashandy et al. (2016) showed that the protective effect of zinc can be attributed to its antioxidant and antiinflammatory properties.

Selenium content of vegetable flours achieved within this study (18.65-31.23 μg/100 g) is comparable to that reported by Lyons et al. (2005) in case of grain (0.5-72 μg/100 g). These authors stated that the variation of selenium content is determined by the selenium content of the soil. Selenium is an essential micronutrient with an important role into human body (thyroid hormone metabolism, cardiovascular health, prevention of neurodegeneration and cancer, and optimal immune responses) (Huang et. al., 2012).

Total polyphenol content

Flours achieved from winemaking by-products and tomato waste flour are potential sources of natural antioxidants. Thus, these are characterized by the total polyphenol content and tomato waste flour contains in addition carotenoids (lycopene and β-carotene). Total polyphenol content of the achieved flours is presented in Figure 5. Total polyphenol content of the achieved flours from winemaking by-products varied in the range 200.15-322.75 mg GAE/g, the maximum value being recorded in case of grape pomace flour. Values of polyphenol content obtained within this study are higher compared to that reported by Tseng and Zhao (2013) for flour obtained from black grape seed and peel, *Pinot Noir* cultivar (67.74 mg GAE/g, flour moisture being 5.63%; flour was obtained by lyophilisation of residues resulted after winemaking, at temperature -55 °C and vacuum of 17.33 Pa).

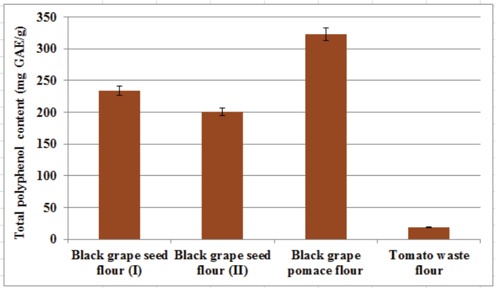


Figure 5. Total polyphenol content of flours achieved from winemaking by-products and tomato waste

At the same time, polyphenol content of the achieved grape seed flour is lower than that reported by Gül et al. (2013), for seed flour of Öküzgözü pomace (552.10 mg GAE/g) and seed flour of Narince pomace (563.27 mg GAE/g). Total polyphenol content of black grape pomace flour (322.75 mg GAE/g) achieved in this study is higher than that reported by Gül et al. (2013), for whole flour of Öküzgözü pomace (236.6 mg GAE/g) and, respectively, for whole flour of Narince pomace (65.93 mg GAE/g). Total polyphenol content of winemaking by-products is influenced by many factors: grape cultivar, climate conditions and culture area, ripening time, processing and storage conditions, as well as the used extraction methods and analytical methods (Lafka et al., 2007).

Phenolic compounds in extracts of grape pomace present antioxidant, anticancerigene and antidiabetes properties (Ruberto et al., 2007; Hogan et al., 2010; Parry et al., 2011; Zhou and Raffoul 2012; González-Centeno et al., 2013), as well as antibacterial activity against *E. coli, L. monocytogenes*, and *S. aureus* (Ozkan et al., 2004; Darra et al., 2012).

Polyphenol content of tomato waste flour (18.76±0.19 mg GAE/g; moisture = 7.64±0.17%) was significantly lower compared to that of the flours achieved from winemaking by-products. Nour et al. (2015) obtained for tomato waste (moisture content = 69.98±0.18%) a total polyphenol content of 0.866 ±0.012 mg GAE/g.

Antioxidant capacity

Antioxidant capacity of the achieved flours is presented in Figure 6.

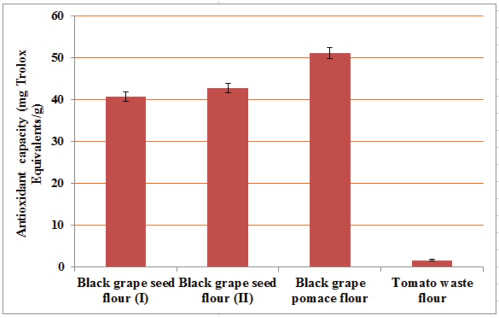


Figure 6. Antioxidant capacity of the flours achieved from winemaking by-products and tomato waste

Antioxidant capacity of flour achieved from winemaking by-products varied in the range 40.75–51.25 mg Trolox Equivalents/g, maximum value being recorded for black grape pomace flour.

Antioxidant capacity of flour achieved from tomato waste was 1.62 mg Trolox Equivalents/g.

For flours achieved from winemaking by-products and tomato waste, between the total polyphenol content and antioxidant capacity it is a linear correlation, regression coefficient R^2 being 0.9559 (Figure 7).

Results are in conformity with those of Ky and Teissedre (2015) which obtained positive correlations between the total polyphenol content and antioxidant capacity (DPPH method) for seed extract and for skin extract, respectively (R^2 = 0.87 for seed extract, R^2 = 0.79 for skin extract).

Lycopene content of the tomato waste flour was 225.92 mg/kg, and ß-carotene content, respectively 16.22 mg/kg. Lycopene content of tomato waste flour was higher than that reported by Nour et al. (2015) for tomato waste (moisture content = 69.98±0.18%): 174.12 mg/kg. ß-carotene content of tomato waste flour achieved in this study was lower than that reported by Nour et al. (2015) 32.66 mg/kg.

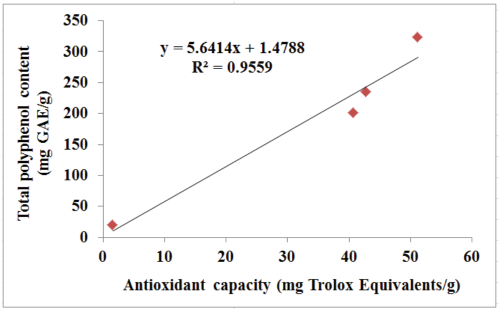


Figure 7. Correlation between the total polyphenol content and antioxidant capacity in case of flours achieved from winemaking by-products and tomato waste

Microbiological analysis

Results of the microbiological analysis of flours achived from tomato waste and winemaking by-products (grape seed, grape pomace) are presented in the Table 2.

Table 2. Microbiological analysis of flours achieved from tomato waste and winemaking by-products

Flour type	Yeast and mold (CFU/g)	*Enterobacteriaceae* (CFU/g)	*Escherichia coli* (CFU/g)	*Salmonella* (in 25g)	Water activity (Aw)
Black grape seed flour (I)	< 10	< 10	< 10	absent	0.338
Black grape seed flour (II)	< 10	< 10	< 10	absent	0.289
Black grape pomace flour	< 10	< 10	< 10	absent	0.274
Tomato waste flour	< 10	< 10	< 10	absent	0.344

Microbiological analysis shown that the achieved flours are in the frame of the provisions of the legislation into force. These flours show low values of water activity (0.274-0.344), which give them microbiological stability.

CONCLUSIONS

Flours achieved from tomato waste and winemaking by-products are important sources of protein, minerals (K, Ca, Mg, Fe, Zn and Se), dietary fibres and bioactive compounds. Thus, black grape pomace flour and black grape seed flour are characterized by total polyphenol content (200.15 mg GAE/g...322.75 mg GAE/g) and tomato waste flour by content of carotenoids (lycopene - 225.92 mg/kg; ß-carotene - 16.22 mg/kg). Also these flours have antioxidant potential being beneficial in a healthy diet for prevention of diseases caused by free radicals. On the other hand, flours achieved in this study are characterized by high dietary fibre content (58.86-66.06%), being important sources to increase the fibre content of foods (bakery products, pastry products, etc.). Increase of the fibre content in case of the sweet flour products is very important because it reduces their glycemic impact on the human body, thus preventing the development of diabetes mellitus and obesity. Also, dietary fibre have an important role in promoting feeling of satiety and detoxification of the human body.

Flours achieved from winemaking by-products and tomato waste can be regarded as functional ingredients and can be used to fortify food products (bakery and pastry products, especially) in order to increase the nutritional and their antioxidant potential.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Research and Innovation, by Nucleu Programme PN 16 46, contract 29 N/2016.

REFERENCES

Abbaspour N., Hurrell R., Kelishadi R., 2014. Review on iron and its importance for human health. J Res Med Sci., 19(2):164–174.

Aghajanzadeh A., Maheri N., Mirzai A., Baradaran A., 2010. Comparison of nutritive value of Tomato pomace and brewers grain for ruminants using in vitro gas production technique. Asian Journal of Animal and Veterinary Advances, 5:43-51.

Aghamirzaei M., Peighambardoust S.H., Azadmard-Damirchi S., Majzoobi M., 2015. Effects of Grape Seed Powder as a Functional Ingredient on Flour Physicochemical Characteristics and Dough Rheological Properties. J. Agr. Sci. Tech., 17:365-373.

Anastasiadi M., Chorianopoulos N.G., Nychas G.-J.E., Haroutounian S.A., 2008. Antilisterial activities of polyphenol-rich extracts of grapes and vinification byproducts. J Agric Food Chem., 57: 457-463.

Bampi M., Bicudo M.O.P., Fontoura P.S.G., Ribani R.F., 2010. Composição centesimal do fruto, extrato concentrado e da farinha da uva-do-japão. Ciência Rural, 40(11):2361-2367.

Bashandy S.A.E.M., Omara E.A.A., Ebaid H., Amin M.M., Soliman M.S., 2016. Role of zinc as an antioxidant and anti-inflammatory to relieve cadmium oxidative stress induced testicular damage in rats. Asian Pacific Journal of Tropical Biomedicine, 6(12):1056–1064.

Borguini R.G., Da Silva Torres E.A.F., 2009. Tomatoes and tomato products as dietary sources of antioxidants. Food Rev Int, 25:313–325.

Bravo L., Saura-Calixto F., 1998. Characterization of dietary fiber and the *in vitro* indigestible fraction of grape pomace. American Journal of Enology and Viticulture, 49(2):135-141.

Burton-Freeman B., Talbot J., Park E., Krishnankutty S., Edirisinghe I., 2012. Protective activity of processed tomato products on postprandial oxidation and inflammation: a clinical trial in healthy weight men and women. Molecular Nutrition and Food Research, 56:622–631.

Campos L.M.A.S., Leimann F.V., Pedrosa R.C., Ferreira S.R.S., 2008. Free radical scavenging of grape pomace extracts from Cabernet Sauvignon (*Vitis vinifera*). Bioresour. Technol., 99:8413–8420.

Chen Z.-Y., Ma K.Y., Liang Y., Peng C., Zuo Y., 2011. Role and classification of cholesterol-lowering functional foods. J. Funct. Foods, 3:61–69.

Choi Y., Lee J., 2009. Antioxidant and antiproliferative properties of –tococtrienol-rich fraction. Food Chem., 114: 1386–1390.

Darra N.E., Tannous J., Mouncef P.B., Palge J., Yaghi J., Vorobiev E., et al., 2012. A Comparative study on antiradical and antimicrobial properties of red grapes extracts obtained from different *Vitis vinifera* varieties. Food Nutr. Sci., 3:1420–1432.

Del Valle M., Cámara M., Torija M.E., 2006. Chemical characterization of tomato pomace. Journal of the Science of Food and Agriculture, 86:1232–1236.

Demirbas A., 2010. Oil, micronutrient and heavy metal contents of tomatoes. Food Chem, 118:504–507.

Eller F.J., Moser J.K., Kenar J.A., Taylor S.L., 2010. Extraction and analysis of tomato seed oil. J Am Oil Chem Soc, 87:755–762.

FAOSTAT. 2012. FAOSTAT agriculture production database.http://faostat. fao.org / site/ 339/default.aspx.

FAOSTAT, 2010.http://faostat.fao.org.

Fernandes L., Casal S., Cruz R., Pereira J.A., Ramalhosa E., 2012. Seed oils of ten traditional Portuguese grape varieties with interesting chemical and antioxidant properties. Food Res. Int., 50:161-166.

Fuentes E., Astudillo L., Gutiérrez M., Contreras S., 2012. Fractions of aqueous and methanolic extracts from tomato (*Solanum lycopersicum* L.) present platelet antiaggregant activity, Blood Coagulation and Fibrinolysis, vol. 23, pp. 109–117.

George B., Kaur C., Khurdiya D.S., Kapoor H.C., 2004. Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. Food Chem, 84:45–51.

González-Centeno M.R., Jourdes M., Femenia A., Simal S., Rosselló C., Teissedre P.-L., 2013. Characterization of polyphenols and antioxidant potential of white grape pomace by-products (*Vitis vinifera* L.). J. Agricult. Food Chem., 61:11579–11587.

Gül H., Acun S., Şen H., Nayır N., Türk S., 2013. Antioxidant activity, total phenolics and some chemical properties of Öküzgözü and Narince grape

pomace and grape seed flours. Journal of Food, Agriculture & Environment, 11(2):28 -34.
Hogan S., Zhang L., Li J., Sun S., Canning C., Zhou K., 2010. Antioxidant rich grape pomace extract suppresses postprandial hyperglycemia in diabetic mice by specifically inhibiting alpha-glucosidase. Nutrition & Metabolism, 7:71.
Horszwald A., Andlauer W., 2011. Characterisation of bioactive compounds in berry juices by traditional photometric and modern microplate methods. Journal of Berry Research, 1:189–199.
Hsu Y.M., Lai C.H., Chang C.Y., Fan C.T., Chen C.T., Wu C.H., 2008. Characterizing the lipid-lowering effects and antioxidant mechanisms of tomato paste. Bioscience, Biotechnology and Biochemistry, 72(3):677–685.
Huang Z., Rose A.H., Hoffmann P.R., 2012. The Role of Selenium in Inflammation and Immunity: From Molecular Mechanisms to Therapeutic Opportunities. Antioxidants & Redox Signaling, 16(7):705-743.
Ky I., Teissedre P.L., 2015. Characterisation of Mediterranean Grape Pomace Seed and Skin Extracts: Polyphenolic Content and Antioxidant Activity. Molecules, 20:2190-2207.
Lachman J., Hejtmánková A., Hejtmánková K., Horníčková S., Pivec V., Skala O., Dědina M., Přibyl J., 2013. Towards complex utilisation of winemaking residues: Characterisation of grape seeds by total phenols, tocols and essential elements content as a by-product of winemaking. Industrial Crops and Products, 49:445– 453.
Lafka T.I., Sinanoglou V., Lazos E.S., 2007. On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chemistry, 104:1206–1214.
Liadakis G.N., Tzia C., Oreopoulou V., Thomopoulos C.D., 1995. Protein isolation from tomato seed meal, extraction optimization. J Food Sci, 60:477–482.
Llobera A., Cañellas J., 2007. Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): pomace and stem. Food Chemistry, 101(2):659-666.
Lyons G.H., Genc Y., Stangoulis J.C.R., Palmer L.T., Graham R.D., 2005. Selenium distribution in wheat grain, and the effect of postharvest processing on wheat selenium content. Biological Trace Element Research, 103:155-168.
Maier T., Schieber A., Kammerer D., Carle R., 2009. Residues of grape (*Vitis vinifera* L.) seed oil production as a valuable source of phenolic antioxidants. Food Chem., 112:551–559.
Martínez-Valverde I., Periago M.J., Provan G., Chesson A., 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). J Sci Food Agric, 82:323–330.
Majzoobi M., Ghavi F.S., Farahnaky A., Jamalian J., Mesbahi G., 2011. Effect Of Tomato Pomace Powder On The Physicochemical Properties Of Flat Bread (Barbari Bread). Journal of Food Processing and Preservation, 35:247–256.
Nagata M., Yamashita I., 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J Jpn Soc Food Sci Technol, 39:925–928.
Ngamukote S., Mäkynen K., Thilawech T., Adisakwattana S., 2011. Cholesterol-Lowering Activity of the Major Polyphenols in Grape Seed. Molecules, 16:5054-5061.
Nour V., Ionica M.E., Trandafir I., 2015. Bread enriched in lycopene and other bioactive compounds by addition of dry tomato waste. J Food Sci Technol, 52(12):8260–8267.
Ozkan G., Sagdic O., Baydar N.G., Kurumahmutoglu Z., 2004a. Antibacterial activities and total phenolic contents of grape pomace extracts. J. Sci. Food Agric., 84:1807–1811.
Ozcan M.M., 2010. Mineral contents of several grape seeds. Asian J. Chem., 22:6480–6488.
Palomo I., Gutierrez M., Astudillo L., Rivera C., 2009. “Efecto antioxidante de frutas y hortalizas de la zona central de Chile”. Revista Chilena De Nutricion, 36:152–158.
Parry J.W., Li H., Liu J.-R., Zhou K., Zhang L., Ren S., 2011. Antioxidant activity, antiproliferation of colon cancer cells, and chemical composition of grape pomace. Food Nut. Sci., 2:530–540.
Papaioannou E.H., Karabelas A.J., 2012. Lycopene recovery from tomato peel under mild conditions assisted by enzymatic pre-treatment and non-ionic surfactants. Acta Biochim Pol, 59:71–74.
Pérez Jiménez J., Serrano J., Tabernero M., Arranz S., Díaz-Rubio M.E., García-Diz L., Goñi I., Saura-Calixto F., 2008. Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. Nutrition, 24(7-8):646-653.
Persia M.E., Parsons C.M., Schang M., Azcona J., 2003. Nutritional evaluation of dried tomato seeds. Poult Sci, 82:141–146.
Ray R.C., El Sheikha A.F., Panda S.H., Montet D., 2011. Anti-oxidant properties and other functional attributes of tomato: an overview. Int J Food Ferment Technol, 1:139–148.
Rockenbach I.I., Rodrigues E., Gonzaga L.V., Fett R., 2010. Composição de ácidos graxos de óleo de semente de uva (Vitis vinifera L. e Vitis labrusca L.). Brazilian Journal of Food Technology, IIISSA.
Ruberto G., Renda A., Daquino C., Amico V., Spatafora C., Tringali C., Nunziatina-De T., 2007. Polyphenol constituents and antioxidant activity of grape pomace extracts from five Sicilian red grape cultivars. Food Chem., 100:203–210.
Savatović S.M., Gordana S., Ćetković G.S., Čanadanović-Brunet J.M., Djilas S.M., 2010. Utilisation of tomato waste as a source of polyphenolic antioxidants. Acta Period Technol, 41:187–194.
Sousa E.C., Uchôa-Thomaz A.M.A., Carioca J.O.B., de Morais S.M., de Lima A., Martins C.G., Alexandrino C.D., Ferreira A.T., Rodrigues A.L.M., Rodrigues S.P., Silva J.N., Rodrigues L.L., 2014. Chemical composition and bioactive compounds of grape pomace (Vitis vinifera L.), Benitaka variety, grown in

the semiarid region of Northeast Brazil. Food Sci. Technol, Campinas, 34(1):135-142.

Toor R.K., Savage G.P., 2005. Antioxidant activity in different fractions of tomatoes. Food Res Int, 38:487–494.

Tseng A., Zhao Y., 2013. Wine grape pomace as antioxidant dietary fibre for enhancing nutritional value and improving storability of yogurt and salad dressing. Food Chem., 1; 138(1): 356-65.

Valiente C., Arrigoni E., Esteban R.M., Amado R., 1995. Grape pomace as a potencial food fiber. Journal of Food Science, 60(4):818-820.

Zhou K., Raffoul J.J., 2012. Potential anticancer properties of grape antioxidants. J. Oncol. Article ID 803294.

Zuorro A., Lavecchia R., Medici F., Piga L., 2013. Enzyme-assisted production of tomato seed oil enriched with lycopene from tomato pomace. Food Bioprocess Technol, 6(12):3499–3509.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

VITAMIN C AND TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY OF FRESH AND PROCESSED FRUITS OF *ARONIA MELANOCARPA*

Luminița CATANĂ[1], Monica CATANĂ[1], Enuța IORGA[1], Adrian Constantin ASĂNICĂ[2], Anda-Grațiela LAZĂR[1], Monica-Alexandra LAZĂR[1], Nastasia BELC[1]

[1]National Research & Development Institute for Food Bioresources, IBA Bucharest, 6 Dinu Vintila Street, District 2, 021102 Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: lumi_catana@yahoo.co.uk

Abstract

There are scientific evidences that a diet rich in fruits and vegetables may reduce the risk to have different chronic diseases. Berries are recommended in a healthy diet as it provides protection against degenerative diseases, cardiovascular diseases and cancer. Fruits of Aronia melanocarpa are rich sources of biologically active compounds, polyphenols (anthocyanins and procyanidins, especially) representing the most important group. Polyphenols are the main substances which give the antioxidant potential of black chokeberry fruits. In this paper are presented results of the performed research for determination of vitamin C and total polyphenol content and antioxidant capacity in case of fresh and processed fruits of Aronia melanocarpa (frozen and dried fruits, juice, jam, compote). Determination of vitamin C was performed by high performance liquid chromatography coupled with high resolution mass spectrometry, using hippuric acid as internal standard. Total polyphenol content was spectrophotometric determined, using Folin-Ciocalteu method, and assessment of the antioxidant capacity was performed using DPPH method. Vitamin C content of the samples taken into study varied in the range 7.25–98.75 mg/100g (minimum value was recorded for jam, and maximum one for fresh, unpasteurized juice). Dried fruits of Aronia registered the highest total polyphenol content (4015.25 mg GAE/100g) and antioxidant capacity (84.45mg Trolox Equivalents/g). Minimum value of antioxidant capacity was recorded for compote of Aronia, 12.25 mg Trolox Equivalents/g, respectively. Taken into consideration that fresh fruits of Aronia are available only a short time period, their processing under diverse forms is of real interest for consumers which can benefit thus of nutritional qualities and antioxidant potential of them.

Key words: *Aronia melanocarpa, fruits, polyphenols, antioxidant capacity.*

INTRODUCTION

Among berries, fruits of *Aronia melanocarpa*, they have gaine recently attention due to the health claims associated with their consumption (Chrubasik et al., 2010; Kokotkiewicz et al., 2010). Black chokeberry (*Aronia melanocarpa* (Michx.) Elliott belongs to the family *Rosaceae* and is native to the North America and Canada, being cultivated in Europe in the early twentieth century (Konić Ristić et al., 2013). Fruits of *Aronia melanocarpa* (Michx.) Elliott are rich sources of biologically active compounds, polyphenols (anthocyanins and procyanidins, especially) representing the most important group. Polyphenols are the main substances which give the antioxidant potential of black chokeberry fruits (Kokotkiewicz et al., 2010). Thus, black chokeberry fruits (*Aronia melanocarpa* (Michx.) Elliott) are an important natural source of cyanidin 3-O-glycoside anthocyanins (cyanidin 3-O-galactoside, cyanidin 3-O-glucoside, cyanidin 3-O-arabinoside, and cyanidin 3-O-xyloside) (González-Molina et al., 2008), quercetin derivatives (Bermúdez-Soto and Tomás-Barberán, 2004), hydroxycinnamic acids (Zheng and Wang, 2003). Total polyphenolic content varies in the range 2-8 mg/100 d.m. and depends on the cultivar, growing conditions and harvesting time (Kähkönen et al., 1999; Hakkinen et al., 1999; Benvenuti et al., 2004; Oszmiański and Wojdyło, 2005; Hudec et al., 2006; Sueiro et al., 2006). Lidija Jakobek et al. (2012) determined polyphenols content in case of three cultivars (‘Viking’, ‘Nero’, ‘Galicianka’) of fruits of chokeberry (*Aronia melanocarpa*) and wild chokeberries, in

Croatia, region Slavonia during two consecutive years (2010 and 2011). Cultivars 'Viking', 'Nero' and wild chokeberries had a similar total polyphenolic content (9,012–10,804 mg kg^{-1} in the first year, 9,361–12,055 mg GAE/ kg FW in the second year).Cultivar 'Galicianka' had a lower total polyphenolic content (8,564 mg GAE/kg⁻FW first year, 8,600 mg GAE/kg FW second year).

Besides polyphenols, fruits of *Aronia melanocarpa* are sources of sugar (10–18%), pectins (0.6–0.7%), the sugar alcohol sorbitol, and parasorboside (Weinges et al.,1998; Niedworok and Brzozowski, 2001; Wolski et al.,2007; Kulling and Rawel, 2008). Also these fruits contain small amounts of fat (0.14% fresh weight), represented especially by linoleic acid glycerides and phosphatidylinositol (Kane et al.,1991; Zlatanov, 1999). Also, Kulling and Rawel (2008) notes that fruits of *Aronia melanocarpa* contain vitamins B (B_1, B_2, B_6, niacin, pantothenic acid), vitamin C (13–270 mg/kg), β-carotene (7.7–16.7 mg/kg), minerals (4.4–5.8 g/kg as ash value), approx. 16–18% of carbohydrates (glucose, fructose, sorbitol), dietary fiber (approx. 55 g/kg) and 1–1.5% of organic acids (malic, quinic, citric).Specific almond flavor of these fruits is given by cyanogenic glycosides – amygdalin (20 mg/ 100 g fresh weight – FW) (Lehmann, 1990; Kullingand Rawel, 2008). Fruits of *Aronia melanocarpa* contain triterpenes (b-sitosterol and campesterol) and over 40 volatile compounds, the most important ones being benzaldehyde cyanohydrine, hydrocyanic acid, and benzaldehyde (Hirviand Honkanen, 1985; Zlatanov, 1999).

Fruits of *Aronia melanocarpa* (black chokeberry) demonstrate antiviral activity against influenza viruses, including an oseltamivir-resistant strain. Ellagic acid and myricetin are two components in fruits of *Aronia*, which give the anti-influenza properties (Parket al., 2013). Also, the polyphenolic-rich *Aronia melanocarpa* juice kills teratocarcinomal cancer stem-like cells, but not their differentiated counterparts (Sharif et al., 2013).

The *in vitro* experiments showed anticoagulant effect of polyphenols-rich extracts from black chokeberry and grape seeds (Bijak et al., 2011). In a pilot study, Maria Handeland et al.(2013) shown that black chokeberry juice (*Aronia melanocarpa*) reduces incidences of urinary tract infection.

Fresh fruits of *Aronia melanocarpa* can be consumed a short period time and thus to benefit by their nutritional qualities and antioxidant potential these fruits are processed under various forms: dried fruits, puree, juice, liqueur, syrup, jam, wine, compote, tea, powder (Chrubasik et al., 2010; Ochmian et al., 2012; Kapci et al., 2013; Šnebergrová et al., 2014). On the other side, fresh fruits of *Aronia melanocarpa*, have sour and astringent taste and therefore consumers prefer juice of *Aronia melanocarpa*, in combination with other fruits, such as, apples, pears and blackcurrant (Lehmann, 1990; Ara, 2002).

Anna Horszwald et al. (2013) studied the influence of drying techniques (spray drying, freeze drying and vacuum drying) on *Aronia* commercial juice, in the temperature range 40-80ºC. It was found that all the obtained powders have a high content in polyphenols, in the range: 27.63±1.38 mg GAE/100 mg DM …34.28±1.77 mg GAE/100 mg DM. Powders obtained by spray drying had the highest content of total flavonoids (5.22±0.32 mg quercetin/100 mg DM), total monomeric anthocyanins (4.80±0.13 mg Cy-3-G/100 mg DM), cyaniding-3-glucoside (21.10±0.63 mg Cy-3-G/100 mg DM) and total proanthocyanidins (59.22±3.69 mg (+)-Catechin/100 mg DM). Also, powders obtained by spray drying had the highest antioxidant capacity (251.34±18.77 μmol Trolox Equivalents/100 mg DM by ABTS; 26.49±2.34 μmol Trolox Equivalents/100 mg DM by TEAC; 248.56±11.06 μmol Trolox Equivalents/100 mg DM by FRAP).

In this paper are presented results of the performed research for determination of vitamin C and total polyphenol content and antioxidant capacity in case of fresh and processed fruits of *Aronia melanocarpa* (frozen and dried fruits, juice, jam, compote).

MATERIALS AND METHODS

Samples

Fresh and dried fruits of *Aronia melanocarpa*, compote, jam and fresh juice of *Aronia* were purchased from private producers. Fresh and

dried fruits of *Aronia melanocarpa* were packed in plastic package. Ingredients of *Aronia* compote, packed in hermetically sealed glass recipients (Twist-off system), 720 mL capacity, were the following: *Aronia* fruits, water, sugar and lemon juice. *Aronia* jam was achieved by concentration of *Aronia* fruits with sugar, with adding of lemon juice and packed in hermetically sealed glass recipients (Twist-off system), 314 mL capacity. Fresh *Aronia* juice was achieved by pressing *Aronia* fresh fruits and packed in hermetically sealed glass recipients (Twist-off system), 330 mL capacity.
Frozen fruits of *Aronia melanocarpa* were achieved within the Pilot Experiments Plant for Fruits and Vegetables Processing from fresh fuits purchased from private producer. Thus, fresh fuits of *Aronia melanocarpa* were sorted, washed and frozen in plastic package (net weight 250 g±3%) at – 18°C.

Methods

Vitamin C content

Determination of vitamin C was performed by high performance liquid chromatography (Accela, Thermo Scientific) coupled with high resolution mass spectrometry (LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap Mass Spectrometer, Thermo Scientific) using hippuric acid as internal standard.

LC conditions:

- Column (Hypersil GOLD aQ, 150 x 2.1 mm, 3 µm);
- Column temperature: 40°C;
- Sample temperature: 4°C;
- Mobile phase A: 990 mL water: 10 mL 1M ammonium formate (aq): 1 mL formic acid;
- Mobile phase B: 990 mL methanol: 10 mL 1M ammonium formate (aq): 1 mL formic acid;
- Flow rate: 0.400 mL/min;
- Injection volume: 25 µL;

MS conditions:

Analyzer: FTMS;
Resolution: 60000;
Ionization mode: ESI-;
Specific ions were: m/z = 175.02438 (for vitamin C) and m/z = 178.05051 (for hippuric acid).

In Figure 1 is presented the calibration curve of vitamin C, achieved in the concentration range 2100-10000 µg/L.

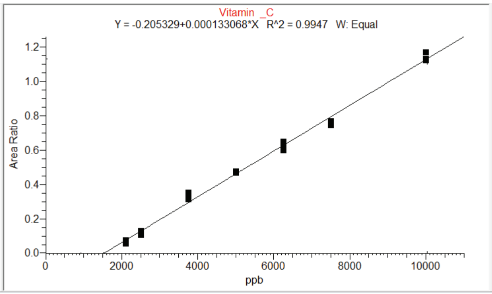


Figure 1.Calibration curve of vitamin C

Total polyphenol content

Total polyphenol content was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range 0-0.20 mg/mL (Figure 2).

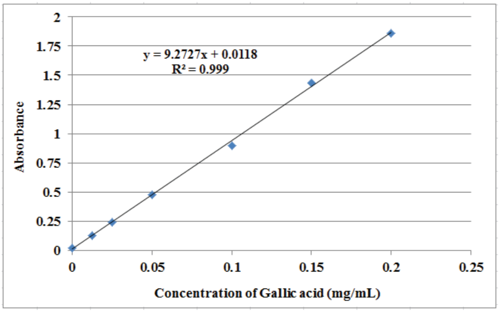


Figure 2. Calibration curve of gallic acid

The extraction of phenolic compounds was performed in methanol:water 50:50, and the absorbance of the extracts was determined at a wavelenght λ = 755 nm. Results were expressed as mg of gallic acid equivalents (GAE) per g product.

Antioxidant capacity

The DPPH scavenging radical assay was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f (Trolox concentration), in the concentration range 0-

0.4375 mmol/L (Figure 3). Results were expressed as mg Trolox Equivalents per g product.

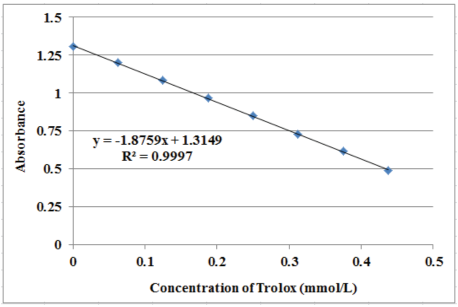


Figure 3. Calibration curve of Trolox

RESULTS AND DISCUSSIONS

Vitamin C content

Vitamin C content of fruits of *Aronia melanocarpa*, fresh and processed (frozen and dried fruits, juice, jam, compote) varied in the range: 7.25–98.75 mg/100 g (Figure 4). Minimum value was recorded for *Aronia* jam, and the maximum one for *Aronia* fresh juice (unpasteurized).

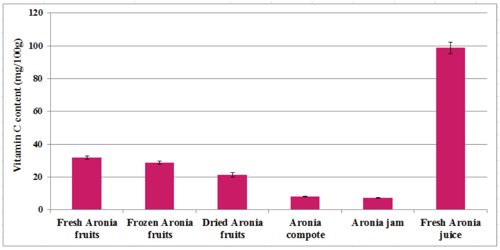


Figure 4. Vitamin C content of fresh and processed fruits of *Aronia melanocarpa*

Vitamin C content of fresh fruits of *Aronia melanocarpa* was 31.85 mg/100 g, and is higher than that mentioned by Kulling and Rawel (2008), 1.3–27 mg/100g and by Karakasova et al. (2014), 17.52 mg/100g, respectively. Also, vitamin C content of frozen fruits of *Aronia melanocarpa* (28.78 mg/100 g) is with 9.64% lower than those of the analyzed fresh fruits in this study, but higher than that mentioned by Karakasova et al. (2014), respectively, 17.15 mg/100 g. Vitamin C content of dried fruits of *Aronia melanocarpa* in this study was 1.4 times higher than that reported by Karakasova et al. (2014), respectively, 15.11 mg/100 g. *Aronia* compote and *Aronia* jam recorded a low vitamin C content (7.96 mg/100 g, respectively, 7.25 mg/100 g), because vitamin C is very sensitive to oxygen and heat treatment. *Aronia* fresh juice analysed in this study is an important source of vitamin C (98.75 mg/100 g). Consumption of about 61 g *Aronia* juice ensure daily requirement of vitamin C for children older than 4 years and adults, respectively (60 mg vitamin C/day). The result obtained for the content of vitamin C of *Aronia* fresh juice is in line with those obtained by Djuricet al.(2015) for *Aronia* juice, obtained from fruits grown on four soil types (91.10-155.20 mg/100 mL). Frei et al. (2012) have shown that dietary supplementation with vitamin C decreased hypertension, endothelial dysfunction, chronic inflammation, and *Helicobacter pylori* infection. At the same time, vitamin C acts as a biological antioxidant that can reduce high levels of oxidative stress and may contribute to chronic disease prevention. Also, based on the performed studies, these authors concluded that 200 mg per day is the optimum dietary intake of vitamin C for the majority of the adult population, to maximize the potential health benefits of this vitamin.

Total polyphenol content

Fruits of *Aronia melanocarpa* are a valuable source of total polyphenols. Total polyphenol content of fresh and processed fruits of *Aronia melanocarpa* is shown in Figure 5. The highest total polyphenol content was recorded for dried fruits of *Aronia* (4015.25 mg GAE/100g), due to the high content of dry matter (89.8%).
The result obtained for the content of bioactive compounds is comparable with that obtained by Kapci et al. (2013) for dried chokeberry (3990±30 mg GAE/100 g, respectively, 5010±40 mg GAE/100 g).

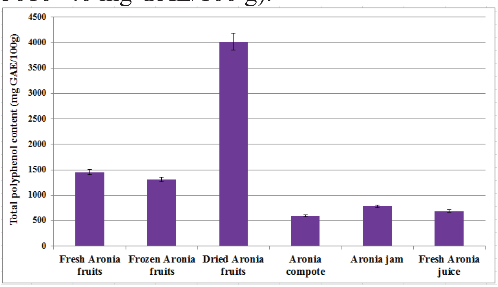


Figure 5. Total polyphenol content of fresh and processed fruits of *Aronia melanocarpa*

Also, total polyphenol content of dried fruits of *Aronia* is about 1.63 times higher than that obtained by Tolić et al. (2015) for chokeberry dried berries (2466±91 mg GAE/100 g of dry matter).

Fresh fruits of *Aronia melanocarpa* in this study had a total polyphenol content (1455.25 mg GAE/100 g) comparable to that reported by Kapci et al. (2013) for fresh chokeberry (1330±3 mg GAE/100 g), but lower than that reported by Ochmian et al. (2012) for four cultivars of chokeberry fruits ('Galicjanka'-2185 mg GAE/100 g; 'Hugin'-2340 mg GAE/100g; 'Nero' - 1950 mg GAE/100 g; 'Viking' - 1845 mg GAE/100 g).

Frozen *Aronia* fruits in this study had a polyphenol content of 1308.75±47.77 mg GAE/100g, comparable to that of fresh fruits.

Aronia compote (containing 55.25% fruits) had the lowest total polyphenol content (590.45±19.18 mg GAE/100 g), lower than that reported by Kapci et al. (2013) for this product (670±3 mg GAE/100 g). Compared to other products, *Aronia* juice had a lower total polyphenol content, this can be explained by the high water content (Shin et al., 2008). Thus, total polyphenol content of *Aronia* fresh juice studied was 688.47±22.75 mg GAE/100 g, higher than that reported by Konić Ristić et al. (2013) in case of commercial chokeberry juice (586±27 mg GAE/100 g), respectively, fresh chokeberry juice (593±33 mg GAE/100 g). Values higher or lower for total polyphenol content of fresh or processed fruits of *Aronia melanocarpa,* reported in the literature, may result by use of various extraction methods and analytical procedures, through application of processing technologies and different conditions, respectively, the differencies between the varieties of these fruits (Denev et al., 2012).

The phenolic compounds are the most important class of bioactive compounds from the fruits of *Aronia melanocarpa,* which are also responsible for many of its medicinal properties (Kulling and Rawel, 2008). Thus, Sikora et al. (2012), in a human study shown that introduction in diet of extract of fruits of *Aronia melanocarpa* had as effects decrease of the lipid levels and significant inhibition of platelet aggregation.

Oprea et al.(2014) have shown that the addition of *Aronia* juice in the normal diet of healthy rats for 10 days, it was correlated with the reduction of values of some markers of oxidative stress and a decrease of blood glucose with 6.85%. Also, administration of *Aronia* juice in case of rats suffering alloxan induced-diabetes resulted in a significant reduction of blood glucose (42.83%).

Recent research undertaken by Daskalova et al. (2015) on animals have shown that treatment with juice of *Aronia melanocarpa* significantly reduced low-density lipoprotein fraction, with pro-aterogenic properties and a decrease of total cholesterol by 16.5%. In case of animals taken into study, dietary supplementation with *Aronia* juice has reduced atherogenic risk and also had a protective effect on the cardiovascular system. However, it was found that *Aronia* juice delay aortic changes that occur with age.

Antioxidant capacity

Antioxidant capacity of fruits of *Aronia melanocarpa*, fresh and processed (frozen and dried fruits, juice, jam, compote) varied in the range:12.25–84.45 mg Trolox Equivalents/g (Figure 6). Minimum value was recorded for *Aronia* compote, and the maximum one for dried *Aronia* fruits. Antioxidant capacity of dried *Aronia* fruits in this study is 2.32 times, respectively, 2.76 times higher than that reported by Kapci et al. (2013) for dried chokeberry (36.3±1.2 mg Trolox Equivalents/g, respectively, 30.5±1.0 mg Trolox Equivalents per g). Antioxidant capacity of frozen *Aronia* fruits (27.39±1.18 mg Trolox Equivalents/g) is with 10.05% lower than that of fresh fruits.

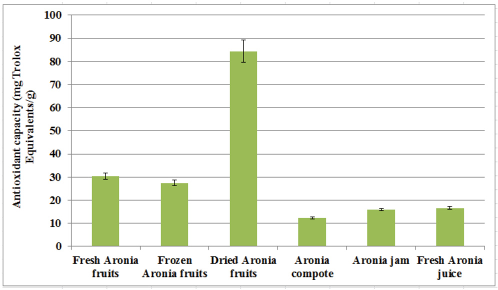


Figure 6. Antioxidant capacity of fresh and processed fruits of *Aronia melanocarpa*

On the other side, antioxidant capacity of fresh *Aronia* fruits is 2.69 times higher than that reported by Kapciet al. (2013) for fresh chokeberry fruit (11.3±0.5 mg Trolox Equivalents/g). Chokeberry fruits have one of the highest *in vitro* antioxidant activities among fruits (Denev et al., 2012).

In this study, *Aronia* jam and fresh *Aronia* juice recorded the following values for antioxidant capacity: 15.88±0.55 mg Trolox Equivalents/g, respectively, 16.55±0.58mg Trolox Equivalents per g. Antioxidant capacity of *Aronia* compote taken into study was 2.55 times higher than that reported by Kapci et al. (2013) for this product (4.8±0.1 mg Trolox Equivalents/g).

Between the total polyphenol content of fruits of *Aronia melanocarpa*, fresh and processed (frozen and dried fruits, juice, jam, compote) in this study and the antioxidant capacity was registered a linear correlation, regression coefficient, R^2, being 0.9988 (Figure 7).

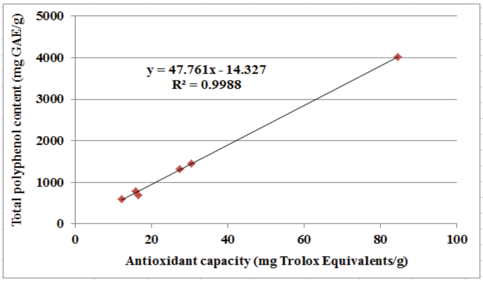


Figure 7. Correlation between total polyphenol content and antioxidant capacity in case of fresh and processed fruits of *Aronia melanocarpa*

Results are in conformity with those of Zheng and Wang (2003), which mentioned a direct correlation between total polyphenol content of fruits of *Aronia* and their antioxidant capacity.

CONCLUSIONS

In this study were evaluated vitamin C content, total polyphenol content and antioxidant capacity (DPPH method) of fruits of *Aronia melanocarpa* fresh and processed (frozen, dried, juice, jam, compote).

Fresh *Aronia* juice recorded the highest vitamin C content (98.75±3,6 mg/100 g). Also, fresh and frozen *Aronia* fruits are valuable sources of vitamin C.

Dried *Aronia* fruits shown the highest total polyphenol content (4015.25±164.63 mg GAE/100 g), followed by the fresh and frozen *Aronia* fruits. Also, fresh *Aronia* juice, *Aronia* jam and compote have an important total polyphenol content.

Dried *Aronia* fruits recorded the highest value of the antioxidant capacity (84.45±4.90 mg Trolox Equivalents/g), followed by fresh and frozen *Aronia* fruits. Fresh *Aronia* juice, *Aronia* jam and *Aronia* compote recorded relatively close values for antioxidant capacity.

Fresh and processed fruits of *Aronia* are valuable because of the content in bioactive compounds and their antioxidant capacity.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Research and Innovation, by Nucleu Programme PN 16 46, contract 29 N/2016.

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, project number PN-II-RU-TE-2014-4-0749.

REFERENCES

Ara V., 2002. Schwarzfruchtige Aronia: Gesund – und bald „in aller Munde“?. Flüssiges Obst,10:653–658.

Benvenuti S., Pellati F., Melegari M., Bertelli D., 2004. Polyphenols, anthocyanins, ascorbic acid, and radicals scavenging activity of Rubus, Ribes, and Aronia.J Food Sci,69:164–169.

Bermúdez-Soto M.J., Tomás-Barberán F.A., 2004. Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. European Food Research and Technology,219(2):133–141.

Bijak M., Bobrowski M., Borowiecka M., Podsędek A., Golański J., Nowak P., 2011. Anticoagulant effect of polyphenols-rich extracts from black chokeberry and grape seeds. Fitoterapia,82:811–817.

Chrubasik C., Li G., Chrubasik S., 2010. The clinical effectiveness of chokeberry: A systematic review. Phytotherapy Research,24:1107–1114.

Daskalova E., Delchev S., Peeva Y., Vladimirova-Kitova L., Kratchanova M., Kratchanov C., Denev P., 2015. Antiatherogenic and Cardioprotective Effects of Black Chokeberry (*Aronia melanocarpa*) Juice in Aging Rats, Evidence-Based Complementary and Alternative Medicine, Volume 2015, Article ID 717439, 10 pages.

Denev P.N., Kratchanov C.G., Ciz M., Lojek A., Kratchanova M.G., 2012. Bioavailability and antioxidant activity of black chokeberry (*Aronia melanocarpa*) polyphenols: *in vitro* and *in vivo* evidences and possible mechanisms of action: a review. Compr Rev Food Sci., 11(5):471–89.

Djuric M., Brkovic D., Miloševic D., Pavlovic M., Curėic S., 2015. Chemical Characterisation of the Fruit of Black Chokeberry Grown on Different Types of Soil. REV. CHIM. (Bucharest), 66(2):178-181.

Frei B., Birlouez-Aragon I., Lykkesfeldt J., 2012. Authors' perspective: What is the optimum intake of vitamin C in humans?.Crit Rev Food SciNutr., 52(9):815-29.

González-Molina E., Moreno D.A., García-Viguera C., 2008. Aronia-enriched lemon juice. A new highly antioxidant beverage.Journal of Agricultural and Food Chemistry, 56(23):11327–11333.

Hakkinen S., Heinonen M., Karenlampi S., Mykkanen H., Ruuskanen J., Torronen R., 1999. Screening of selected flavonoids and phenolic acids in 19 berries.Food Res Int,32:345–353.

Handeland M., Grude N., Torp T., Slimestad R., 2014.Black chokeberry juice (Aronia melanocarpa) reduces incidences of urinary tract infection among nursing home residents in the long term a pilot study. Nutrition Research., 34:518-525.

Hirvi T., Honkanen E., 1985. Analysis of the volatile constituents of black chokeberry (*Aronia melanocarpa*). J Sci Food Agric,36:808–810.

Horszwald A., Andlauer W., 2011. Characterisation of bioactive compounds in berry juices by traditional photometric and modern microplate methods.Journal of Berry Research,1:189–199.

Horszwald A., Heritier J., Andlauer W., 2013.Characterisation of Aronia powders obtained by different drying processes. Food Chemistry,141:2858–2863.

Hudec J., Bakoš D., Mravec D., Kobida L., Burdova M., Turianica I., Hluše J., 2006. Content of phenolic compounds and free polyamines in black chokeberry (*Aronia melanocarpa*) after application of polyamine biosynthesis regulators.J Agric Food Chem,54:3625–3628.

Jakobek L., Drenjančević M., Jukić V., Šeruga M., 2012. Phenolic acids, flavonols, anthocyanins and antiradical activity of "Nero", "Viking", "Galicianka" and wild chokeberries.Scientia Horticulturae,147:56–63.

Kane M.E., Dehgan B., Sheehan T.J., 1991. In vitro propagation of Florida native plants: *Aronia arbutifolia*. Proc Fla State HortSoc,104:287–290.

Kapci B., Neradová E., Čížková H., Voldřich M., Rajchl A., Capanoglu E., 2013. Investigating the antioxidant potential of chokeberry (Aronia melanocarpa) products.Journal of Food and Nutrition Research,Published online 23 October 2013.

Kähkönen M.P., Hopia A.I., Vuorela H.J., Rauha J., Pihlaja K., Kujala T.S., Heinonen M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem,47:3954–3962.

Karakasova L., Babanovska-Milenkovska F., Stojanova M.,Karakasov B., 2014.Comparison of Qualities Properties of Fresh, Frozen and Solar Dried Chokeberry Fruits, 25th International Scientific-Experts Congress on Agriculture and Food Industry, DOI: 10.13140/RG.2.1.4506.1209.

Kokotkiewicz A., Jaremicz Z., Luczkiewicz M., 2010. Aronia plants. A Review of traditional use, biological activities, and perspectives for modern medicine.Journal of Medicinal Food,13:255–269.

Konić Ristić A., Srdić-Rajić T., Kardum N., GlibetićM.,2013.Biological activity of *Aronia melanocarpa* antioxidants pre-screening in an intervention study design.Journal of the Serbian Chemical Society,78(3):429–443.

Kulling S.E., Rawel H.M., 2008. Chokeberry (*Aronia melanocarpa*) - a review on the characteristic components and potential health effects.Planta Medica, 74:1625–1634.

Lehmann H., 1990.Die Aroniabeere und ihre Verarbeitung. FlüssigesObst, 57:340–345.

Niedworok J., Brzozowski F., 2001. The investigation of a biological and phytotherapeutical properties of the *Aronia melanocarpa* E anthocyanins [in Polish].Post Fitoter,1:20–24.

Ochmian I., Grajkowski J., Smolik M., 2012.Comparison of Some Morphological Features, Quality and Chemical Content of Four Cultivars of Chokeberry Fruits (*Aronia melanocarpa*).Not Bot Horti Agrobo, 40(1):253-260.

Oprea E., Manolescu B.N., Fărcăşanu I.C., Mladin P., Mihele D., 2014. Studies Concerning Antioxidant And Hypoglycaemic Activity of *Aronia melanocarpa* Fruits.FARMACIA, 62, 2.

Oszmiański J., Wojdyło A., 2005. *Aronia melanocarpa* phenolics and their antioxidant activity.Eur Food Res Technol,221:809–813.

Park S., Kim J.I., Lee I., Lee S., Hwang M.W., BaeJ.Y., Heo J., Kim D., Han S.Z., Park M.S., 2013. *Aronia melanocarpa* and its components demonstrate antiviral activity against influenza viruses.Biochemical and Biophysical Research Communications,440:14–19.

Sharif T., Stambouli M., Burrus B., Emhemmed F., Dandache I., Auger C., Etienne-Selloum N., Schini-Kerth V.B., Fuhrmann G., 2013.The polyphenolic-rich *Aronia melanocarpa* juice kills teratocarcinomal cancer stem-like cells, but not their differentiated counterparts. Journal of Functional Foods,5:1244-1252.

Shin Y., Ryu J., Liu R.H., Nock J.F., Watkins C.B., 2008. Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit. Postharvest Biol Technol., 49:201–9.

Sikora J., Broncel M., Markowicz M., Wojdan K., Chahalubinski M., Mikiciuk-Olasik E., 2012. Short-term supplementation with *Aronia melanocarpa* extract improves platelet aggregation, clotting and fibrinolysis in patients with metabolic syndrome. Eur J Nutr, 51(5):549–56.

Šnebergrová J., Čížková H., Neradová E., Kapci B., Rajchl A., Voldřich M., 2014.Variability of characteristic components of Aronia.Czech J. Food Sci.,32:25–30.

Sueiro L., Yousef G.G., Seigler D., De Mejia E.G., Grace M.H., Lila M.A., 2006. Chemopreventive potential of flavonoid extracts from plantation-bred and wild *Aronia melanocarpa* (black chokeberry) fruits. J Food Sci, 71:480–488.

Tolić M.T.,Jurčević I.L., Krbavčić I.P., Marković K., Vahčić N., 2015.Phenolic Content, Antioxidant Capacity and Quality of Chokeberry (*Aronia melanocarpa*) Products. Food Technol. Biotechnol., 53(2):171–179.

Weinges K., Schick H., Schilling G., Irngartinger H., Oeser T., 1998.Composition of an anthocyan concentrate from *Aronia melanocarpa* Elliot—X-ray analysis of tetraacetylparasorboside. Eur J Org Chem, 1:189–192.

Wolski T., Kalisz O., Prasał M., Rolski A., 2007. Black chokeberry-*Aronia melanocarpa* (Michx.) Elliot-the rich source of antioxidants [in Polish]. Post Fitoter, 3:145–154.

Zheng W., Wang S.Y., 2003. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. Journal of Agricultural and Food Chemistry, 51(2):502–509.

Zlatanov M.D., 1999. Lipid composition of Bulgarian chokeberry,black currants and rose hip seed oil.J Sci Food Agric,79:1620–1624.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRESENT STATUS AND FUTURE PROSPECTS OF GEOTHERMAL ENERGY USE FOR GREENHOUSE HEATING IN TURKEY

Hasan Huseyin OZTURK

Cukurova University, Faculty of Agriculture Engineering of Agricultural Machineries and Technologies, 01330 Adana, Turkey

Corresponding author email: hhozturk@cu.edu.tr

Abstract

In order to obtain the highest yield of the expected product are grown in greenhouses, it is necessary to heat the greenhouse during periods of low temperatures. Conditions of our country, heating costs are one of the most important factors affecting the profitability of the greenhouse. Greenhouse heating costs vary depending on the product type, growing season and the region, accounted for 40% and 80% of the total cost. Due to the high costs of fossil fuels used for heating greenhouses heating applications cannot be done on a regular in many greenhouse of our country, heating is done only to protect plants from frost. Greenhouse heating applications, utilization of alternative energy sources instead of fossil energy sources is a priority need in order to today's energy assets and to protect the environment. In this study; current situation and problems of geothermal greenhouses in Turkey were assessed and the necessary suggestions were made to improve the utilization of geothermal resources in the greenhouse heating.

Key words: *geothermal energy, greenhouse heating, strategy development.*

INTRODUCTION

In the world of today's industry, the usage of energy and energy resources have crucial value. While the amount of natural resources (especially, fossil fuel resources) has been decreasing, the damage to the natural environment as many type of environmental pollutions has been increasing. Additionally, the technical improvements for the energy conversion can not be carried out as effective as needed. In order to determine the level of future energy production and consumption in developed and developing countries, there are many factors to be considered, such as population growth, economic productivity, consumer habits and technological advances. The style of energy sectors management will play an important role for the future of energy production, consumption and distribution. Careless use of energy resources and their scarcity, resulting unwanted side effects, so energy consumption must be planned and evaluated carefully and accurately.

Geothermal energy is of vital importance in terms of preventing environmental problems like greenhouse effects and acid rains arising from using and consuming fossil fuels. This is primarily because of natural superiority of geothermal energy as of environment when compared with other energy types. On the other hand, important developments have been achieved in terms of solving possible environmental problems that could occur as a result of geothermal energy use. This, in turn increased the importance of geothermal energy with regards to environment. Geothermal energy being one of our domestic resources must be evaluated in preference to other resources in order to decrease the dependency to petroleum in meeting our country’s gap in energy and to prevent foreign currency loss. Geothermal energy is an inexhaustible energy resource as others like hydraulic, solar and wind energy resources. For that reason, when compared with fossil energy resources that are certainly exhaustible, geothermal energy resources are long-lasting and inexhaustible energy resources.

Bertani (2016) has analyzed the major activities carried out for geothermal electricity generation An increase of about 1.8 GW in the five year term 2010-2015 has been achieved (about 17%), following the rough standard linear trend of approximately 350 MW/year. Lund and Boyd (2015) reviewed the worldwide applications of geothermal energy for direct utilization. The report is based on country update papers received from 70 countries and regions of which 65 reported some direct utilization of geothermal energy. The thermal energy used is 592.638 TJ/year (164.635 GWh/year), about a 39.8% increase over 2010, growing at a compound rate of 6.9% annually. The distribution of thermal energy used by category is approximately 55.2% for ground-source heat pumps, 20.2% for bathing and swimming (including balneology), 15% for space heating (of which 89% is for district heating), 4.9% for greenhouses and open ground heating, 2.0% for aquaculture pond and raceway heating, 1.8% for industrial process heating, 0.4% for snow melting and cooling, 0.3% for agricultural drying, and 0.2% for other uses. Energy savings amounted to 352 million barrels (52.8 million tons) of equivalent oil annually, preventing 46.1 million tons of carbon and 149.1 million tons of CO_2 being released to the atmosphere, this includes savings for geothermal heat pumps in the cooling mode (compared to using fuel oil to generate electricity). Considerable work has been conducted on geothermal fields in Turkey and the application of geothermal energy for district heating with respect to efficient and economic use of energy for sustainable development (Mertoglu et al., 2003). Comprehensive studies have been performed on geothermal energy use in agricultural production systems. Greenhouse heating with geothermal energy has been investigated by several authors (Rafferty 1986; Popovski 1988; Bakos et al., 1999; Popovski and Vasilevska, 2003). Optimization of geothermal energy use in agriculture is reflected in two ways, i.e. an increase in productivity at the existing level of energy inputs or conserving the energy without affecting the productivity. In many studies the influence of the direct energy input on energy efficiency was analyzed. Few publications have dealt with the problems related to geothermal energy use in greenhouses. Therefore, in the present study the present status and future prospect were analyzed to develop the geothermal energy use in greenhouse cultivation.

In greenhouse heating applications, using alternative energy resources rather than fossil energy resources is a primary necessity for conserving our energy wealth and preventing the environmental pollution. Some of the alternative energy resources that are used in greenhouse heating are; solar energy, geothermal energy and low temperature heat energy from the wastes of industrial facilities. In this study, the existing situation and problems of geothermal greenhouses in Turkey were evaluated and necessary proposals were given to increase the use of geothermal energy resources in greenhouses.

GEOTHERMAL ENERGY AND GREENHOUSE SECTOR IN TURKEY

Geothermal Energy Potential of Turkey

Turkey is located in the Alpine-Himalayan organic belt is an important region in terms of geothermal resources. In terms of geothermal resources, it is among the first seven countries in the world. Due to its location on Alpine Tectonic Zone, Turkey has a significant potential for geothermal energy. Our country is on the very effective zone in the point of tectonic with graben in the west, basin regime in the middle, compressive tectonic in east and North Anatolian Fault in the north. As a result of fracture and weakness zones and magma actions that reach from these zones to the shallow depths within the shell and/or the surface of earth, magmatic and volcanic events occur. Geothermal systems are developing with the help of geological and meteorological phenomena as a geothermal fluid. Theoretical and determined geothermal energy potential in our country are summarized in Table 1.

Table 1. Potential of Turkey's Geothermal Energy (Ozturk, 2015)

Energy	Theoretical potential	Determined potential
Electricity	4 500	200
Thermal energy	31 100	2 250

The areas containing high-temperature geothermal fluids are located in the west part of Turkey due to the grabens resulting the young tectonic activities. With the effect of volcanism and faulting low and medium temperature fields are located Central Anatolia, Eastern Anatolia and north side of Turkey with the North Anatolian Fault.

Geothermal Energy Use

Although it varies depending on the fluid temperature and regions conditions, geothermal energy use can be examined under two general categories:

1) Electricity generation
2) Direct use of geothermal energy

Heat exchanger systems, as wellhead and borehole heat exchangers, can vary in design depending on the area of the feature. The efficiency, continuity or success of the heating system is based on used accordance with technology. Geothermal water that contains chemical materials at a level causing them not to be directly used and whose heat exchangers and heat energies are transferred into supply network water clean enough to utilize, should be removed from the surroundings in order to avoid environmental pollution.

The benefits provided by the direct use of geothermal energy are:

- ✓ Conversion efficiency is high.
- ✓ Can be utilized in low temperature geothermal resources.
- ✓ Can be utilized from wells for research purposes
- ✓ Project implementation period is short.
- ✓ Drilling costs are cheaper in shallow depths.
- ✓ Geothermal fluid can be transported to long distances.

Depending on the geothermal fluid's chemical properties, heating systems show significant differences. If geothermal fluid does not cause problems as chemical content property, it can be used directly circulated through radiators and proper pipes system in radiator surface. However, if the fluid contains too much minerals and is likely to cause chemical problems (scaling, corrosion and so on), heat of the fluid is transferred to water with low chemical content (water used in city supply networks) via heat exchanger. Thus, the heating provided by the heated water does not cause problems in the system.

The direct use of geothermal energy can be divided into four groups:

1) Housing and workplaces
2) Industrial applications
3) Agriculture and related fields
4) Thermal and health tourism

Greenhouse Sector in Turkey

Greenhouse sector requires high investment and industrial activity. According to TurkStat data (TUİK, 2016), in Turkey in 2015, it has a total of 66,400 hectares of greenhouse area. Total greenhouse area of 30,900 hectares of plastic greenhouses, constitutes 8097 hectares of glass greenhouses. Greenhouse areas are more concentrated in southern provinces. The biggest reason is that southern provinces are warmer than in other cities in the winter. According to the Greenhouse Registration System (OKS), in Turkey, 48% of the greenhouse enterprises have the areas between 2-3 acres in 2013. The ratio of the greenhouse enterprises that have area more than 10 acres is only 1.8%. There are 9000 acres of greenhouses with modern conditions. In modern greenhouses, the average size is 27 acres (Ozturk et al., 2015).

A significant portion of the crops grown in greenhouses are the vegetables (95%) and the least portion is constituted by the fruits (5%). Between the vegetable species that grown in greenhouses, tomato production takes first place with the amount of 50%; cucumber, watermelon, green pepper and eggplant follow it. Production share of other vegetables such as melon, bean, zucchini and lettuce is gradually increasing. The most important type of vegetable, cultivation in low plastic tunnels is watermelon. In our countries greenhouse agriculture, widely produced fruits are banana, strawberry, grapes and nectarines. Cut flower production is increasing rapidly in Turkey and it is made in the regions of Marmara, Aegean and Mediterranean. In Turkey, with the 6.5 million tons of greenhouse production, 10 million TL GDP was provided in 2014 (Ozturk and et al., 2015).

GEOTHERMAL ENERGY USE FOR GREENHOUSE HEATING IN TURKEY

The Importance of Geothermal Energy in Heating Greenhouses

To provide optimal environmental conditions in terms of quality, quantity and development time of the products grown in greenhouses, mainly used for the out-of-season produce, it is required heating in the winter’s cold period and ventilation in summer’s hot period. Keeping under control the ambient temperature, that has an effect mainly on the yield and quality of plant growth and development, is an important factor in greenhouse technology. To obtain maximum yield expected from the products cultivated in greenhouses, it is necessary to heat the greenhouse during periods of low temperatures. Even though it is required excess quantity of energy to heat the greenhouses in winter and summer seasons in Northern European countries, only the needed heating applications are not made enough during cold winter nights due to the suitable ecological conditions at most of the Mediterranean countries. In this case, negations are encountered, regarding the quality, quantity and harvest time of the product.

By controlling the climate conditions, it made greenhouse to spread wider in a year when the agricultural production process is the most important issue in the production of heating. In our countries conditions, heating costs are one of the most important factors affecting the profitability of the greenhouse. In greenhouse enterprises, heating costs depend on growing season, region and product type and they constitute 40% to 80% of the total cost (Ozturk, 2015). Due to the high costs of fossil fuels used for heating greenhouses, a regular heating can't be done in many greenhouses in the our country and the protecting plants from frost heating is only done. Not to be done regularly heating bring some concerns together such as low yield, limitations on the types of production, necessity of using drugs and hormones for agricultural struggle. However, a sufficient heating yield to provide the temperature at which the plant needs can increase by 50-60%. For this reason, in heated greenhouses using geothermal energy, required temperature is provided more economically for plant growth and fertilization and the relative humidity of greenhouse indoor air is controlled with the necessary ventilation, diseases can't occur and yield can increase.

According to the Ninth Development Plan (2007-2013) Mining Special Commission Energy Raw Materials Subcommittee of the Geothermal Working Group Report, including existing state and 2013 projections about Turkey's geothermal electricity generation and geothermal heating, it is stated that a qualification of geothermal energy to be emphasized in particular. In the same report, it is indicated that the use of geothermal energy for electricity generation is limited yet and there has been a steady increase in consumption for heating purposes in recent years, so 635 acres of geothermal heated greenhouse presence, has been targeted as 5000 acres in 2013. But according to the Ministry of Food, Agriculture and Livestock Protected Registration System (OCS), as of September 2013, Turkey's geothermal greenhouse uses of assets has reached to a total of 3202 hectares in 10 provinces. Although increased significantly in recent years, but reached the 64% of the target.

In Tenth Development Plan (2014-2018) Mining Special Commission Geothermal Working Group Report, greenhouse warming target is determined as 600 hectares for 2018 and 1500 hectares for 2023. The use of geothermal resources for heating in greenhouses has to be addressed as an innovation that provides economic and environmental benefits for the agricultural sector. The process of adoption of innovation is a multivariate and complicated process related on the one hand with innovation and on the other hand the systems and individuals that used the innovation. Before studies will be launched to increase the geothermal greenhouse area, first individual characteristics and resources of the target audience, its communication channels, time and social environment to be examined when considering. Besides, increases in national and international food prices increased transfers of the capital from non-agricultural sector to agriculture sector by reversing the situation. In addition, growing interest to the alternative energy resources made geothermal greenhouse investments attractive, especially using modern production technology (hydroponics etc.). Environment, making an agricultural production that does not harm human and animal health, preservation of natural resources, traceability and sustainability and manpower using knowledge in greenhouse activities for ensure reliable product supply has become the most important factor. However, investments initiated by parties who aren't making feasibility studies before starting agricultural activities couldn't be reached to desired goals and leads to the formation of excess capacity for the country. To this end, education-extension and publicity studies and planning of human resources, that ensure required capacity at all parties from production to marketing, are required.

Greenhouse Areas Heated with Geothermal Energy in Turkey

Greenhouse heating applications made with geothermal energy in Europe began in Hungary and Yugoslavia in the 1960s. In our country, while heating greenhouses have gained great importance in the utilization of geothermal energy more effectively, applying technical and economic aspects are faced with some problems. Geothermal heating is one of the places where the most widely used of geothermal resources; geothermal greenhouse heating is becoming increasingly important in Turkey. Greenhouse heating system with geothermal energy was applied for the first time in Turkey in the 0.45 hectares areas of the Denizli-Kızıldere. To more accurately and efficiently take advantage of geothermal resources, low enthalpy will need to make use of advanced technology.

According to the 2013 OKS records in Turkey, greenhouses heated by geothermal energy fields are given in Table 2. In 10 provinces in 3202 decares area, is carried out under cover production using geothermal energy. Almost half of these areas are located in Izmir (24.48%) and Manisa (23.42%). While maximum business is located in Kutahya with 46 business, it is followed by Sanlıurfa and Denizli provinces with 26 business. As shown in Table 2, a very small part of the overall greenhouse is heated with geothermal energy in Turkey. While the value of apparent heat capacity that geothermal drilling issued is 3000 MW in 2002, it is reached to 7000 MW with an increase of 230% in 2012. A 10-year period between 2002-2012 years, our greenhouse space heated by geothermal energy has increased by 406% (Hasdemir et al., 2014).

Distance between geothermal resources and businesses vary too much between businesses. The most important factor in the transport of hot water is the temperature of resources. High temperature water can be used in heating by moving to longer distances. Hot water used for heating is used by bringing from the wells average 541 m away (Hasdemir et al., 2014). Greenhouse businesses use hot water from geothermal wells in heating by exchanger system or directly circulating in greenhouses. 67.21 % of greenhouse businesses use hot water directly and 32.79% of them use heat exchanger system (Hasdemir et al., 2014). Hot water taken into greenhouse largely use as a space heating on soil. Only one company was seen heating with underground pipes (Table 3).

Table 2. Greenhouse Areas Heated Through Geothermal Energy (Hasdemir et al., 2014)

Provinces	Number of business	Greenhouse area (da)	The ratio in total geothermal greenhouse area (%)
Afyon	6	358	11.18
Aydın	17	173	5.40
Denizli	26	456	14.24
İzmir	15	784	24.48
Kırşehir	1	97	3.03
Kütahya	46	125	3.90
Manisa	7	750	23.42
Nevsehir	1	61	1.91
Şanlıurfa	26	373	11.65
Yozgat	2	25	0.78

Table 3. Heating Systems in Greenhouses Heated with Geothermal Energy

Properties	Number of business	Ratio (%)
Direct heating	18	67.21
Heat exchanger use	40	32.79
Above ground heating system	121	99.18
Below ground heating system	1	0.82
Reinjection	21	17.21
Refiner	41	33.61
Dumping to the land	60	49.18

67.21 % of available geothermal resources have additional greenhouse warming potential. Despite the availability of geothermal fluid brought to surface, the reasons why the businesses cannot do additional greenhouses are stated by themselves as follows: 32.93 % for insufficiency of land, 29.27% for not being able to acquire official permit, 19.51% insufficiency of capital, 4.88% for high costs, 2.44 % for marketing problems, 1.22% insufficiency of workforce and technical reasons, 8.54% for other reasons.

RESULTS AND DISCUSSIONS

Legislation on Geothermal Resources Prioritizes Electricity Production

Due to problems that Turkey face in energy sector, the tendency is towards using local resources and for this reason certain subsidies and incentives were provided particularly in recent years to investors in these areas. In this context Article 6, Paragraph one of "*Law on the Use of Renewable Energy Resources for Electricity Production"* states that "For those who has the production license subject to Renewable Energy Resources Support Mechanism which are or will be in business from the enforcement of this law on 18/5/2005 to 31/12/2015, the prices annexed to this law in annex 1 as a chart will be applied for 10 years". This means that purchase of the energy produced by using renewable resources is guaranteed for 10 years. It is pledged in the document that the electricity produced through renewable resources is going to be purchased at the rates of 10.5 $cent/kWh, and for 13.2 $/kWh in cases where local manufacturing is utilized (Ozturk, 2015).

Additionally, due to the fact that licenses are provided for a limited period of 3+1 years and the high fluid temperatures figured after drilling creates a tendency in investors to launch Geothermal Energy Plants (GEP). Although there is no limitation for other areas, the uncertainties in these areas causes investors to steer their investments in electricity production. The investments are directed to electricity production because, when they want to use geothermal energy in greenhouse sector there is uncertainty in the number of people and the area that will utilize greenhouse sector; when they want to use it in household heating, there is the uncertainty of potential problems with local administrations and the people who will use it. Because that the GEP's in operation before end of 2015 will receive guaranteed purchases from the states, the owners of geothermal resources want to complete their energy investments before 2015. They plan their investments in other areas after completion of energy investments. When the temperatures of geothermal resources are not sufficient for energy production, they can be utilized in greenhouses. The owners of geothermal plants may use the remaining heating potential in greenhouses after energy production, if they wish to do so.

The Licensed Geothermal Areas

The biggest obstacle for geothermal resources to be used in greenhouses is that after a process of sale of geothermal areas through procurements, the owners and the people who would like to use these areas in greenhouses are different. The owners do not encounter problems when they would like to use the resources in greenhouses, however when others would like to use it the same way, they are either reluctant to give these for greenhousing or they sell it for too high prices. Because that geothermal investments are in abundance and the number of people who will perform greenhouse sector or that the areas where greenhouses are established are separated increasing costs of deploying geothermal energy makes it further difficult to use in greenhouse sector. In cases where the greenhouses would be established collectively closer to the geothermal resources, the transfer of geothermal energy to these areas will be less costly and thus the owners approach may change. In this regard, the purchasers of geothermal plants need to know the proximity of those greenhouses of whose owners are willing to use geothermal heating in their greenhouses. This way it will be easier for owners of geothermal energy to calculate the annual operation costs and determine the price of energy for greenhouse sector. For this reason, the greenhouses should be collectively established near the geothermal resources.

When preparing the law for the use of geothermal resources in other areas, it is necessary to consider giving initiative to the local administrations close to geothermal resources to define priorities based on needs of the region and to give them the opportunity to utilize the geothermal resources. Even if there is a change in law now, this will be beneficial for the areas up for future procurement. In case there is no change in current law, introducing a provision in the contract of procurement for the use of geothermal energy in other areas would provide ground for more efficient utilization of the geothermal resources. There is currently no sanction or enforcement for the owners of the plants to use geothermal energy other than electricity production. Another difficulty is the determination of the price per hectares of geothermal energy provided by the owners. If the pricing is not reasonable, this is a problem for geothermal greenhouse sector. Measures are necessary in order to determine the price of geothermal energy and to avoid future conflicts.

Due to the fact that the geothermal areas are licensed, the initiative to decide on where to use the energy is held by owners. It is necessary to allow the social use by local administrations of blocked areas which surround the geothermal resources for their protection. It is absolutely necessary to make changes for local communities to use the geothermal resources. It does not appear to be a fair process to preserve the use of these areas which were held by the state until today only to the use of renters.

Finding Land and Geothermal Resources for Greenhouses

As a result of the use of geothermal resources after the introduction of the legislation, the number of entrepreneurs willing to invest in this area is increasing. Entrepreneurs mostly face difficulties to find areas for and to use geothermal energy for greenhouses. Sometimes they find the area for greenhouse sector but cannot find the energy, and sometimes otherwise. Consequently, it is important to keep entrepreneurs in business by promoting geothermal energy owners in investing in other areas and in greenhouse sector through incentives, subsidies or low-interest credit opportunities.

The owners of geothermal resources may face problems in finding greenhouse areas when they want to invest themselves. This is because there is not enough land owned by state or available land is private properties. When they want to buy land for investing in greenhouse sector, the prices may raise up to 10 fold. This affects geothermal greenhouse sector negatively.

The Cost of Searching and Developing Geothermal Resources

The high costs of geothermal investments make it more difficult for it to be utilized in geothermal greenhouse sector. Due to the fact that the drill operations for geothermal resource searching is costly and that the cost of re-injection wells and re-injection is high, investors tend to lean towards geothermal power plants which have lower costs and quick returns. The heavy metals, particularly the phototoxic boron in plants, have negative consequences for the environment which increases the costs due to compulsory reinjection and this effects greenhouse sector negatively.

Greenhouse Costs

Costs of modern technological hydroponic culture greenhouses change depending on the technology used and the cover material. The costs of PC sided and PE roofed steel construction and computer controlled greenhouses starts from 50-60 €/m², and of steel construction glass greenhouses starts from 75-100 €/m² (Ozturk, 2015).

Because that the current modern hydroponic culture greenhouses in Turkey are imported or patented outside of the country and that almost all of automation systems are imported as well, the costs are high. Thus it is important to develop local technologies in greenhouse automation systems. The high costs of greenhouses effects greenhouse sector and hence geothermal greenhouse sector negatively.

Problems Arising from Agriculture based Specialization Organized Industry Zones

The best means of greenhouse sector is through the establishment of Agriculture Based Specialization Organized Industry Zones in terms of more efficient use of geothermal source, healthier production, monitoring potential of production and healthier organization of marketing. There are problems both in legal and local terms in establishment of these Zones. These problems also negatively influence geothermal greenhouse sector

Published in 10.10.2009 at the Official Gazette numbered 27402 "The Application Regulation on Establishment of Agriculture Based Specialization Organized Industry Zones" was executed by Ministry of Science Industry and Technology and Ministry of Agriculture and Rural Affairs later in 12/03/2012 by Ministry of Food Agriculture and Livestock. In order for greenhouse sector to be performed in larger areas, the elimination of the rule regarding the requirement of 75% treasury land in which Zones to be established for greenhouse sector will pave the way for greenhouse sector effectively. However, the future of rural population whose lands are already expropriated is concerning.

Many producers in this situation will opt for migrating to cities in which they will encounter adaptation and economic problems. In this case, the high income groups will invest in greenhouse sector in these Zones. This is why the geothermal resources have to be planned in a way to positively influence the lives of local communities rather than negatively. In the regulation, people whose lands were expropriated should be given priority places in Zones. Because that these are small-scale family businesses, it is difficult for them to invest in greenhouses with their own assets, thus they should be provided with 7-10 years credit opportunities without repayment in the first 2-3 years or grants.

As a result of the developments in greenhouse sector in recent years, it is largely modern technology hydroponic culture greenhouse sector. When these modern greenhouses grow, the costs decrease. This is why, if there is a plan to establish such a greenhouse for economic investment the lower limit should be at least an area of 2-2.5 hectares. It is stated that for a Zone to be established there should be at least 50 hectares of land and 30 persons should be involved in the project.

For a modern greenhouse sector in Zones to be economically meaningful it is necessary either to increase the area of Zones required or to decrease the number of people required to be involved. It is necessary to determine the sizes of lands depending on the technology applied in greenhouses.

However, if there is hydroponic culture greenhouses in Zones than the greenhouses should be planned at the minimum 2-2.5 hectares. It is important to take into account the clear water and the geothermal resource to be utilized in greenhouses in the process of establishment.

Administration Shares of Facilities Utilizing Geothermal Energy

In the relevant law and regulations, it is stated that 1% equivalent of the gross production of those facilities directly or indirectly utilizing geothermal fluids or gasses stated in the Law will be collected as administrative cost. In this situation, 1% equivalent of the gross production of greenhouses utilizing geothermal energy will be paid as Special Administration cost. 1% equivalent is a considerably high payment for geothermal greenhouses. 1% equivalent payment should be for those businesses which utilize the geothermal resources directly and whose sole input is geothermal energy such as electricity production, CO_2 producer facilities. For those facilities utilizing geothermal energy as a secondary or tertiary source, 1% should either not be paid or decreased.

Turkish Development Law and the Law on Construction Inspection

Greenhouses are regulated under 'Turkish Development Law No. 3194' and subjected to license. While greenhouses without geothermal heating are subjected licensing process of Ministry of Food Agriculture and Livestock and State Hydraulic Works, the ones with greenhouse heating go through licensing process of below institutions:

1. Provincial Directorate of Culture and Tourism,
2. Provincial Directorate of Science, Industry and Technology,
3. Provincial Directorate of Food, Agriculture and Livestock,
4. State Hydraulic Works,
5. Provincial Directorate of Environment and Urbanization,
6. Special Provincial Directorate of Administration,
7. Electricity Distribution Company,
8. Provincial Gendarmerie Command
9. Directorate of Highways

Legal Status of Greenhouse Sector

New legal arrangements are needed to regulate the legal status of greenhouse sector also addressing all the aspects of it; from planning to construction and production.

This can be resolved through new regulations to be introduced by The Ministry of Food

Agriculture and Livestock. Other relevant Laws and Regulations applicable to this subject also should be revised.

Greenhouses should be in accordance with 1:100.000 scaled Provincial Environment Oder Plans and excluded from the scope of the *Development Law*. Greenhouses should be also exempted from the Law on Construction Inspection. These greenhouses should be classified as agricultural structures under the new regulations to be implemented and all pre-construction and post-construction works or inspections, including planning and authorization should be centralized under the mandate of a single authority. The best implementation approach for this matter would be centralizing all these processes under the mandate of the Ministry of Food Agriculture and Livestock.

Government Incentives Available for Geothermal Greenhouse Production

Modern greenhouse production systems are expected to face a rapid growth with increased use of technology which improves yield and profitability. In order to achieve the targets of agricultural sector in the future, appealing incentives are offered by the government for greenhouse investments in our country. Greenhouse production is the third biggest sector of our country in terms of food and agriculture investments prominent after livestock and dairy sector. In 2012, greenhouse production was listed as the most invested sector following livestock and other relevant sectors.

Despite the diverse geothermal capacity that our country possess, as a result of the insufficient levels of use, geothermal sector have been endorsed for incentives. In this regard applicable legal arrangements are as follows:

- The Communiqué on "Support for Agriculture Investments (No#2011/9)" published in the Official Gazette No#27871 dated 11.03.2011 within the scope of Support Programs for Rural Development Investments.
- "The Regulation Amending the Regulation on Pasture Land" promulgated in the Official Gazette No#27857 dated 28.02.2011.
- The Communiqué on "The Procedure and Principles to be Pursued Regarding Public Domain Allotments for Technology, Geothermal Greenhouses and Organic Farming Investments" published in the Official Gazette No#26511 dated 03.05.2007.
- The regulation for "Implementation of Agro-Industries and Organized Greenhouse Sites" published in the Official Gazette No#27402 dated 10.11.2009.
- "The General Communiqué on National Estate" published in the Official Gazette No#27211 dated 26.04.2009 (I/N: 324)
- "The General Communiqué on National Estate" published in the Official Gazette No#27901 dated 10.04.2011 (I/N: 335)
- "The Decree on Concerning State Aids in Investments" published in the Official Gazette No#27290 dated 16.07.2009.

Within the framework of "Support Program for Rural Development Investments", renewable energy integrated greenhouse investments projects with a budget up to 3 million at max are remunerated by 50% through Financial State Aids. Furthermore, within the scope of the National Real Estate General Communiqué (number:352) published in the Official Gazette No# 27211 dated 26/04/2009, state land are allocated for entrepreneurs that shall invest on geothermal greenhouse systems. Accordingly, with respect to the changes made on the Law on Pastures, pasture lands are also available for geothermal greenhouse investment allocations.

Technology Transfer in Geothermal Greenhouses in Turkey and Sources of Agricultural Extension Information

Technology transfer, extension and adoption of innovations in geothermal greenhouses in Turkey are realized through different extension methods such as agricultural extension programs, demonstrations, field days, farmer meetings, farmer courses, farmer examination trips, incentive competitions, conferences, panels, other similar activities and mass extension media produced to be used in these activities. The information sources that are effective in decision making processes of enterprises using and not using geothermal energy resources are classified in three groups. These information sources were determined as; informal sources like the producer himself, his neighbors or relative and formal sources like Provincial/District Agricultural Directorates, agricultural consultants and technical staff of the enterprises and pesticide and fertilizer distributors that provide input to agriculture and the media organizations. While the enterprises that are using geothermal resources are using formal information sources, the enterprises not using geothermal resources are using informal information sources.

CONCLUSIONS

In accordance with the Protected Cropping Registry System of Ministry of Food Agriculture and Livestock, as of September 2013, total land allocated for protected cropping with geothermal heating in Turkey reached 3202 da in 10 different provinces. Although a significant increase can be noted in recent years, only 64% of the set targets could be achieved. As indicated in the report of Geothermal Working Group of Specialization Commission for Mining, under Tenth Development Plan (2014-2018); the target for geothermal greenhouse heating was determined as 600 ha for year 2018 and it was indicated as 1500 ha for year 2023. It is fundamental to consider geothermal greenhouse heating systems as an innovation that endeavor economic and environmental benefits for agriculture sector. The adoption process of innovation is while eminently related to the innovation itself it is also a sophisticated process encompassing the system that the innovation will be implemented and the users. Before implementing the work connected with the efforts to increase greenhouse areas with geothermal heating, it is essential to consider individual features of the target group and resources including communication channels, time and social environment.

Organized Greenhouse Sites offering cost-efficient, secure, traceable, competitive, modern and planned protected cropping with strong brand equity, clustered in regions which are rich in terms of geothermal resources (such as Afyonkarahisar, Aydın, Denizli, Diyarbakır, İzmir, Kırşehir, Konya, Kütahya, Manisa, Şanlıurfa and Yozgat) are aimed to be established. Having a geothermal capacity of 31,500 MW, Turkey possess enough resources in terms of reaching the targeted 600 ha of land for year 2018 and 1500 ha of geothermal heated greenhouse area for year 2023. However, said resources should be efficiently managed and strategies should be in place to ensure the establishment of sustainable geothermal greenhouses.

Making use of alternate energy resources instead of fossil fuels for greenhouse heating systems is a priority concern in terms of conserving the energy resources of today's World and preventing environmental pollution. In Turkey, although effective use of geothermal energy for greenhouse heating has become more important, there are still some tackles resulting from the implementation in terms of economic and technical aspects. As geothermal heating known as a common way of adopting geothermal resources, geothermal greenhouse heating has become significantly important in Turkey. Recycled liquid injected back into Geothermal Power Plants for reinjection can also be used for greenhouse heating and legal arrangements should be made in this regard. It is fundamental to consider geothermal energy resources as an innovation that endeavor economic and environmental benefits for agriculture sector.

REFERENCES

Bakos G.C., Fidanidis D., Tsagas N.F., 1999. Greenhouse heating using geothermal energy. Geothermics, 28:759-765.

Bertani R. 2016. Geothermal power generation in the world 2010–2014 update report. Geothermics 60:31-43

Hasdemir M., Hasdemir M., Gül U., Yasan Ataseven Z., 2014. Geothermal Greenhouses in Turkey. Project Final Report, TEPGE Publication No: 227, ISBN: 978-605-4672-60-8.

Lund J.W., Boyd T., 2015. Direct utilization of geothermal energy 2015 worldwide review. Geothermisc, 60:66-93.

Mertoglu O., Bakir N., Kaya T., 2003 Geothermal applications in Turkey. Geothermics, 32:419-428.

Ozturk H.H., 2015. Geothermal Greenhouses. Umuttepe publications, Publication No: 150, ISBN: 978-605-5100-56-8, Kocaeli, Turkey.

Popovski K., 1988. Factors influencing greenhouse heating and geothermal heating systems. Geothermics, 17:173-189.

Popovski K., Vasilevska S.P., 2003. Prospects and problems for geothermal use in agriculture in Europe. Geothermics, 32:545-555.

Rafferty K., 1986. Some considerations for the heating of greenhouses with geothermal energy. Geothermics, 15:227-244.

TUIK., 2016. Turkish Statistic Foundation, Ankara, Turkey.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CHEMICAL CONSTITUENTS OF THE ESSENTIAL OIL OF *ARTEMISIA SANTONICA* L. (*ASTERACEAE*) ECOTYPES FROM ROMANIA

Monica Luminiţa BADEA, Aurelia DOBRESCU, Elena DELIAN, Ioana Marcela PĂDURE, Liliana BĂDULESCU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: badea.artemisia@gmail.com

Abstract

Artemisia L. (Asteraceae) genus includes many species being the largest genus from Anthemideae tribe, as well as one of the largest genus from Asteraceae family. Of these species, A. santonica is a perrenial herb or small shrubs and have numerous uses in various field such as medicine, nutrition and industry (phytochemicals). Due to its characteristic features in terms of chemical composition, and their usefulness, the purpose of this paper is to present recent results regarding the chemical composition of essential oil extacted by hidrodistillation from A. santonica. The plant material were collected from different areas of Romania. The qualitative and quantitative essential oil analysis was performed by gas chromatography/mass spectrometry (GC/MS). The results expressed as percentage show the presence of chemical compounds represented in majority by α -thujona, β -thujona, borneol, eucalyptol and camphor.

Key words*: essential oils, Artemisia, Asteraceae, chromatography, ecotype.*

INTRODUCTION

Artemisia L. (*Asteraceae*) genus comprised almost 500 species being the largest genus from *Anthemideae* tribe and one of the largest genus from *Asteraceae* family (Bohm and Stuessy, 2001; Watson et al., 2002). Based on ethnobotanic studies, most species of *Artemisia* genus have numerous uses in traditional medicine, nutrition and industry (phytochemicals).
Artemisia santonica is a perennial herb or small shrubs, frequently aromatic. Stems 20-60 cm, cylindrical, woody in the lower part, glabrous or grey-tomentose. The leaves are usually grey-tomentose or glabrescent, rarely white, inferior leaves short petiolate, leaves slightly auriculate at the basis, with ovate lamina- 2-3 pennately-divided, with cu norow lacinia, rounded at the top; capitula small, numerous, usually pendent, in racemose, paniculate or capitate inflorescences, rarely solitary. Involucral bracts are disposed in few rows. Receptacle is flat to hemispherical without scales, sometimes hirsute. Florets are yellow, all tubular. Achenes is obovoid, absent or sometimes a small scarious ring (Figure 1).
The ethnobotanical and bio-chemical studies revealed the fact that *A. santonica* extracts proved to be benefic antioxidant effects, also in the anthelmintic treatment, in disorders of the digestive and urinary tract, and as poultices and infusions calms the cough and the cephalalgia. In the French kitchen is used as a spice. The extracts of *Artemisia* used in large doses can become toxic (Badea and Delian, 2014; Githiori et al., 2006; Tandon et al., 2011).
The objective of this study was to bring new information regarding the composition of essential oil extracted from the species *A. santonica*, collected from different areas (ecotypes) of Romania.

MATERIALS AND METHODS

Artemisia santonica L. plants were harvested during full blossoming, from natural populations in different areas of Romania: Slănic-Prahova (Prahova County), Ocna-Sibiului (Sibiu County), Slobozia (Ialomița County) and Plopu (Tulcea County).
The essential oil extraction was realized from *Herba*. Fresh herbal parts of the collected plants were subjected to hydrodistillation for 3h using a Singer-Nickerson equipment to produce oil. The separation and identification of components has been carried out using an Agilent gas chromatograph, equipped with quadruple mass spectrometer detector. A capillary column DB-5

(25 m length x 0.25 mm i.d. and 0.25 µm film thickness) and helium as carrier gas were used. The initial oven temperature was 60°C, then rising to 280°C at a rate of 4°C /min. The NIST spectra bank was used for to identify the essential compounds, which were verified with the Kovats indices.

Figure. 1. Morphological aspect of *Artemisia santonica* L.

RESULTS AND DISCUSSIONS

GC-MS analyses of the oils were carried out according to a procedure that has been described above. The yield and composition of the essential oils of *Artemisia santonica* ecotypes are presented in Table 1.

The essential oil extracted from the *A. santonica* ecotypes, coming from the 4 ecotypes (Slănic-Prahova, Ocna-Sibiului, Slobozia and Plopu) contained a number of different chemical constituents: 15 (in Plopu) and 33 (in Ocna-Sibiului). Only five substances are common to all 4 ecotypes: α-thujona, β - thujona, eucalyptol, terpinen-4-ol and germacrene D, also three of them are considered as majority.

After Burzo (2008), the major compounds at the species *A. santonica* are represented by eucalyptol, camphor, cis-verbenol, borneol.

Comparing the substances founded in the majority of the essential oils extracted from the 4 ecotypes, was established that α-thujona had a limit of variation between 25.73% (Ocna-Sibiului) and 70.09% (Slobozia) compared to β-thujona whose variation limit was between 5.22% (Plopu) and 17.28% (Slobozia).

The eucalyptol was determined in proportion of 1.64% at the ecotype Plopu and 9.08% at the one from Ocna-Sibiului.

Those three majority substances (α-thujona, β-thujona and eucalyptol) varied widely, depending on the ecotype. Thereby, their total amount had the lowest value at the ecotype Ocna-Sibiului (45.12%), had intermediate values at the ecotypes Slănic-Prahova (58.32%) and Plopu (71.24%), and the highest value was measured at the ecotype Slobozia (91.31% from the total of the substances identified).

A small variation was measured in the case of terpinen-4-ol (0.50% at the Plopu ecotype and 2.73% at the one from Ocna-Sibiului) and at germacren D (0.14% at the Plopu ecotype and 1.14% at the one from Slobozia).

The camphor and the borneol are not present in composition of the essential oil from Slobozia ecotype, but these substances were quite well represented in the composition of the essential oil that came from the other three ecotypes. Thus, the camphor level varied between 4.71% (Plopu) and 24.65% (Slănic-Prahova) and the borneol values ranged between 1,57% at the Slănic-Prahova and 24.13% at the ecotype Ocna-Sibiului (Table 1).

Major compounds from this species (α-thujona, β-thujona, borneol and camphor) represent 66.99% from the total of the substances identified in the essential oil from the Ocna-Sibiului, 77.87% from the Slănic ecotype, 87.37% -Slobozia and 94.43% from the Plopu ecotype.

Table 1. Composition of essential oil extracted from 4 ecotypes of *Artemisia santonica* (% from total identified substances)

Compounds	Slănic-Prahova	Ocna-Sibiului	Slobozia	Plopu
ethyl methyl butyrate	0.39	-	0.15	-
dimethyl metilen ciclohexane	-	-	0.48	-
santona -trien	0.09	-	-	-
tricyclen	0.30	-	-	-
α-pinene	0.15	0.54	-	-
camphene	4.70	1.93	-	0.35
sabinene	0.31	1.63	0.44	-
β-pinene	0.28	0.33	-	-
dehydro -cineole	-	0.27	-	-
β-felandren	-	-	-	0.14
1- octen-3-ol	-	-	-	0.18
α-terpinene	0.18	0.71	-	-
cimene	-	1.23	0.70	0.12
β -cimen	0.76	-	-	-
eucalyptol	6.67	9.08	3.94	1.64
artemisia ketone	2,87	-	-	-
y-terpinene	-	0,26	0,28	-
terpinolene	-	0,30	-	-
α-terpinolene	0.09	-	-	-
α-thujona	44.80	25.73	70.09	64.56
β-thujona	6.85	10.31	17.28	5.22
izothujol	-	0.33	-	0.22
crisantenona	0.43	-	-	-
trans-pinocarveol	-	1.28	0.42	0.33
camphor	24.65	6.82	-	4.71
sabina ketone	-	0.30	-	-
borneol	1.57	24.13	-	19.94
pinocarvone	0.55	-	-	-
terpinen-4-ol	0.66	2.73	0.66	0.50
α-thujenal	-	0.23	-	-
α-terpineol	-	0.53	-	0.18
myrtenol	0.61	0.67	-	0.28
isobornil formate	-	-	0.25	-
cumin aldehide	0.06	0.11	0.10	-
carvone	0.13	-	-	-
trans-chrysantenyl acetate	0.07	0.51	-	-
bornyl acetate	0.24	0.29	-	-
myrtenil acetate	3.53	0.96	0.16	-
α-copaen	-	0.17	-	-
trans -pinocarvil acetate	-	-	0.14	-
germacrene D	0.39	0.91	1.14	0.14
β-selinene	-	0.19	-	-
elixen	0.15	-	0.92	-
spatulenol	-	0.11	-	-
cariofilen oxid	-	0.15	-	-
tau-muurolol	-	0.53	-	-
α-cadinol	-	0.21	-	-
selinene-4-ol	-	0.33	-	-

Concerning the minority of chemical compounds, can be affirmed that were registered significant differences between the ecotypes. Thereby, the α-pinene, the camphene, the β-pinene, the α-terpinene, the trans-chrysanthenyl acetate, the bornyl acetate and the myrtenyl acetate were determined in plants found in the ecotypes Slănic-Prahova and Ocna-Sibiului, while the sabinene and the cumin aldehyde were founded in the essential oil extracted from the plants located in the Slanic-Prahova, Ocna-Sibiului and Slobozia ecotypes, missing at the ecotype Plopu.

The γ-terpinene has been identified at the plants from Ocna-Sibiului and Slobozia ecotype missing at the plants from Slanic-Prahova and Plopu.

The compounds ethyl methyl butyrate and elixen, have been determined in the essential oil resulted from the plants of the Slănic-Prahova and Slobozia, but were missing from the Ocna-Sibiului and Plopu ecotypes (Table 1).

Also, there were a minority of chemical compounds specific for each ecotype, as much:

- the essential oils of Slănic Prahova ecotypes contained santonia-trien, tricyclen, β-cimen, crisantenona, artemisia ketone, pinocarvone;
-the plants from Ocna-Sibiului ecotype contained dehydro-cineole, terpinolene, sabina ketone, α-thujenal, and β-selinene.
- in Slobozia ecotype contained dimethyl metilen ciclohexane, cimene, isobornyl formate, trans-pinocarvyl acetate, myrtenil acetate;
- the plants from Plopu ecotype contained α-felandren and 1-octen-3-ol;

The plants of *A. santonica* L. are considered halophiles, the essential oil extracted from the plants grown on a saline soil, with a high concentration of NaCl (Ocna-Sibiului and Slănic-Prahova) recorded a greater number of chemical components (27-33), comparative with the others two ecotypes analyzed (15-16).

CONCLUSIONS

The main substances identified in *Artemisia santonica* are represented by α-thujona, β-thujona, borneol, eucalyptol and camphor.

The total of the substances that were a majority varied between: 66.99% at the ecotype Ocna Sibiului, 77.87% at Slănic-Prahova, 87.37% at Slobozia and 94.43% at Plopu ecotype, from the total of all substances identified.

The chemical composition of essential oil extracted from the species *Artemisia santonica* varied depending on the ecotype.

ACKNOWLEDGEMENTS

We warmly thank to Prof. dr. Burzo Ioan from the Faculty of Horticulture, Bucharest

REFERENCES

Badea M.L., Delian E., 2014. *In vitro* antifungal activity of the essential oils from *Artemisia* spp. L. on *Sclerotinia sclerotiorum,* Romanian Biotechnological Letters 19(3): 9345-9352.

Badea M.L., Zamfirache M.M., 2011. Anatomical research on *Artemisia santonica* and *Artemisia scoparia* (*Asteraceae*). Analele ştiinţifice ale Universităţii „Al. I. Cuza" Iaşi, s. II a. Biologie vegetală , LVII (2): 21-24.

Bohm B. A. and Stuessy T.F., 2001. Flavonoids of the Sunflower Family (*Asteraceae*) Springer-Verlag Publishing House, Wein, 831pp.

Bremer K., 1994. *Asteraceae,* cladistics and classification*,* Timber Press: Portland. Carnegie Institution of Washington: Washington, DC.

Burzo I., Ciocârlan V., Delian E., Dobrescu A., Bădulescu L.,2008. Researches regarding the essential oil composition of some *Artemisia* L. species. Analele Stiinţifice ale Universităţii „Al. Ioan Cuza″ Iaşi - Secţiunea II a. Biologie Vegetală, Editura Univ.Alex.Ioan Cuza Iaşi, LIV(2): 86-91.

Ciocârlan V., 2009. Flora ilustrată a României, Editura Ceres, Bucureşti.

Dr.Schar-Common Name: Santonica | Scientific Name: Artemisia Santonica available on: *http://doctorschar.com/archives/artemisia-santonica/*

Githiori J.B., Athanasiadou S., Thamsborg S.M., 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants, 139(4): 308–320.

Săvulescu T., Ghişa E., Grinţescu I., Guşuleag M., Morariu I., Nyarady E.I., Prodan I., 1964. Flora R.P.R., Editura Academiei Române, vol.IX, Bucureşti.

Tandon V., Yadav A.K., Roy B., Das B., 2011. Phytochemicals as cure of worm infections in traditional medicine system, Emerging Trends in Zoology: 351–378.

Tutin T. G., 1976. *Artemisia* L.: In: Tutin T. G., Burges N. A., Chater A. O., Edmondson J. R., Heywood V. H., Moore D. M., Valentine D. H., Walters S. M., Webb D. A. (Eds.)*,* "Flora Europaea" (Plantaginaceae to Compositae and *(Rubiaceae*), Cambridge: Cambridge University Press, 4: 178-186.

Valles J. and McArthur E.D., 2001. *Artemisia* Systematics and Phylogeny: Citogenetic and Molecular Insights. In: McArthur, E. D.; Fairbanks, Daniel J., comps. 2001. Shrubland ecosystem genetics and biodiversity: proceedings; 2000 June 13–15; Provo, UT. Proc.RMRS-P-21. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Watson L. E., Bates P. L., Unwin M. M., Evans T. M., and Estes J.R., 2002. Molecular phylogeny of Subtribe *Artemisiinae (Anthemideae: Asteraceae),* including *Artemisia* and its allied and segregate genera. BMC Evolutionary Biology. 2: 17(12 PP) Springer-Verlag: Wien.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPARISON OF THE COSTS OF MATING DISRUPTION WITH TRADITIONAL INSECTICIDE APPLICATIONS FOR CONTROL OF CODLING MOTH IN APPLE ORCHARDS IN TURKEY

Orkun Baris KOVANCI

Uludag University, Faculty of Agriculture, Department of Plant Protection, Gorukle campus, 16059, Bursa, Turkey

Corresponding author email: baris@uludag.edu.tr

Abstract

Mating disruption is an alternative control tactic that prevents male insects from finding females, resulting in lower pest density and less crop damage. However, the relatively high cost of mating disruption compared to the conventional chemical control may be an impediment to its adoption by growers worldwide. Therefore, this study aimed at comparing the costs of mating disruption with insecticides for control of codling moth, Cydia pomonella L., in apple orchards in Turkey in 2013 and 2014. Experimental orchards consisted of semi-dwarf 'Gala' and 'Fuji' apple cultivars. Codling moth populations, the number of insecticide applications and management costs varied between cultivars and years. When averaged over cultivars, mating disruption decreased the total number of sprays for apple pest complex by 40.70% and 56.60% in 2013 and 2014, respectively. All control costs related to the number of insecticide sprays, the application of pheromone dispensers, labour, machinery, fuel and other pheromone-based expenses such as pest monitoring were analyzed. Based on partial budgeting analysis, mating disruption treatments lowered insecticide and machinery costs but increased labour costs compared with conventional treatments. The cost of mating disruption ranged from $193.70 higher than the conventional treatment in cv. 'Gala' in 2013 to $ 96.00 less than the conventional treatment in cv. 'Fuji' in 2014. A break-even analysis showed that a price decrease of 22.22% and 70.37% for pheromone dispensers would be required to convince growers to use mating disruption in cvs. 'Gala' and 'Fuji' in 2013, respectively. However, the cost of mating disruption programme was similar or less than a conventional insecticide programme in 2014. The reduction of initial pest density, as well as the improvement of biological control, could lead to the development of more cost-effective and efficacious mating disruption programmes in subsequent years.

Key words: *Cydia pomonella L., chemical control, cost analysis, economics, pheromones.*

INTRODUCTION

Codling moth *(Cydia pomonella* L.) is a key deciduous fruit pest that poses a great economic threat to growers worldwide. Apart from apple, it also attacks pear, walnut, and quince (Witzgall, 2008). Damage is done by larvae, which feed on the fruit skin and bore deeply into the fruit. Larvae can cause up to 100% damage in untreated orchards (Elkins et al., 2005). Damaged apples are culled before packing, making it more challenging for growers to maintain profitability.

Codling moth management mainly relies on chemical control. Two to three cover sprays are commonly used to target hatching eggs and larvae of the first generation codling moth (Kovanci, 2015). In Turkey, growers begin to spray insecticides such as diflubenzuron, methoxyfenozide, novaluron or thiacloprid with an air-blast sprayer at 150 degree-days after the biofix in pheromone traps. Likewise, second generation codling moth larvae are also treated with at least two insecticide sprays at 800 degree-days.

However, the increasing resistance of codling moth populations to insecticides, coupled with adverse effects on beneficial insects, have led to control failures in the field (Reyes et al., 2007). In addition to high costs of chemicals, spraying equipment and gas, insecticide resistance has already increased control costs with traditional insecticide applications. Additional indirect costs of pesticide use on the human health and environment remain to be evaluated. Thus, there is a great need for alternatives to chemical control.

Mating disruption is an alternative control tactic that prevents male insects from finding females, resulting in lower pest density and less crop damage (Cardé and Minks, 1995). In this technique, growers apply large quantities of pheromone dispensers to cause no or delayed mating by disrupting chemical communication in the orchard. Mating disruption has proven to be a viable alternative method to control key pests including the codling moth, Oriental fruit moth (*Grapholita molesta* Busck.) and pink bollworm (*Pectinophora gossypiella* Saunders) (Cardé and Minks, 1995).

Unlike chemical control, mating disruption has no known toxicity or adverse effects on natural enemies so far. Besides, it has the potential to reduce or eliminate the need for insecticide treatments. However, pheromone dispensers are more costly than insecticides. Placement of pheromone emitters is labour intensive since they must be hand-deployed high in the canopy in most orchards (Elkins and Shorey, 1998).

In this context, the paper presents a detailed cost analysis of mating disruption versus conventional chemical control in 2013 and 2014. The objective of this study was to determine if the adoption of mating disruption would be financially feasible for control of codling moth in apple orchards in Turkey.

MATERIALS AND METHODS

Data on codling moth population, insecticide and pheromone use and cullage rates were collected on two apple orchards in 2013 and 2014. The orchards were located in Deydinler village of Inegol town (40.03° N, 29.53’ E) near Bursa, Northwestern Turkey. One orchard contained semi-dwarf cv. ‘Gala trees’ on M.9 rootstock, while the other orchard contained semi-dwarf cv. ‘Fuji trees’ grafted on M.26 rootstock. Trees were trained with the tall spindle at a spacing of 1.7 x 3.3 m, resulting in approximately 1750 trees per ha. Fuji trees had central leader training with 2 x 4.5 m spacing (1100 trees/ha).

Each orchard was 10-ha in size and allocated into two 5-ha plots for mating disruption and chemical control. Conventional insecticide plots were separated by at least 500 m from mating disruption plots.

In the chemical control plots, Fuji trees received a total of 5 and 6 applications for control of codling moth in 2013 and 2014, respectively, whereas Gala trees had only a total of 3 and 4 sprays. A total of two cover sprays were made with diflubenzuron (Dimilin 48 SC, Hektas, Turkey) at 20 ml/ 100 l water (300 ml/ha) against eggs and larvae of first generation codling moth on May and June in both years. Depending on years, one to four thiacloprid (Calypso 240 OD, Bayer, Turkey) at 40 ml/ 100 l water (400 ml/ha) and methoxyfenozide (Prodigy 240 SC, Dow Agro Sciences, Turkey) treatments at 60 ml/100 l water (400 ml/ha) were applied to control second generation codling moth in July and August in 2013 and 2014.

In mating disruption plots, thiacloprid was applied once to control codling moth in mid-May before pheromone application. Pheromone dispensers (Isomate C Plus®, Sumitomo, Turkey) were hung on trees by hand at 1000 dispensers/ha in early-June each year. Each dispenser was baited with 190 mg of codling moth pheromone containing (E, E) - 8, 10-Dodecadien-1-ol. Four pheromone traps (Pherocon CM, Trece, USA) were used for monitoring codling moth adults in each plot.

Points of indifference between mating disruption and insecticide sprays at different prices for pheromone dispensers were determined by break-even analysis. For this purpose, cullage records were obtained from growers in both years. Data was evaluated using t-tests at 95% confidence level (Williamson et al., 1996).

In order to predict percent damage by codling moth larvae, fruit assessments were made by picking 100 fruit randomly from each of the 10 trees per treatment. Fruits with stings, entries and live larvae were counted and analyzed by ANOVA. The presence of codling moth larvae was confirmed by cutting fruits.

The technique of partial budgeting was used to examine the cost differences between two management alternatives for codling moth. The US dollar ($) amounts were provided using an exchange rate of 1TL = 0.5$ at the time of the study. The price per unit of pheromone and insecticide materials were multiplied by their amount of use to calculate the final cost per ha.

In budget assumptions for machinery, predicted fuel and lubrication costs of a 60 h.p. tractor with an air-blast sprayer were at $ 2.00 per hour of operation. The same sprayer had estimated repair costs at $ 1.00 per hour. An average spray may need 1 machine hour per ha. Hence, fuel and lube cost for spraying in insecticide plots were calculated as 1 hour x \$2.00/hour x number of sprays. Similarly, one insecticide spray in mating disruption plots costs \$ 2.00 (1 hour x \$ 2.00/hour x 1 spray).

The labour for machinery and placing pheromone dispensers in the orchard were also calculated. Machine labour cost for insecticide sprays was calculated using the following formula, where the value 1.10 represents the need for 10% more man hours to refill or clean the sprayer (Williamson et al., 1996): Machine labour cost = Machine hours x 1.10 x \$ 2.00/hour x number of sprays.

The labour for hanging pheromone dispensers at 1000 dispensers/ha by hand was estimated to cost \$ 4.00 per hour. The installation takes about 5 hours per ha to complete. Thus, pheromone applications would cause an estimated increase of \$ 20.00 in labor costs to deploy pheromone dispensers.

Each pheromone dispenser cost \$ 0.27, with a total of \$ 270.00 per ha. Other pheromone related expenses included monitoring of codling moth and other pests with pheromone traps or visual inspection. A pheromone trap package for codling moth costs \$ 30 per ha. Fixed or overhead costs were considered unchanged between operations using mating disruption and conventional insecticide.

RESULTS AND DISCUSSIONS

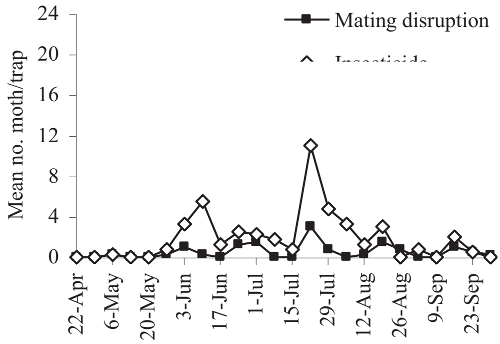
Mean codling moth catch in pheromone traps averaged across cultivars in 2013 and 2014 in Bursa, Turkey is shown in Figure 1.

Codling moth populations were higher in 2014 than in 2013. Pheromone traps in mating disruption plots caught fewer moths compared with insecticide plots in both years.

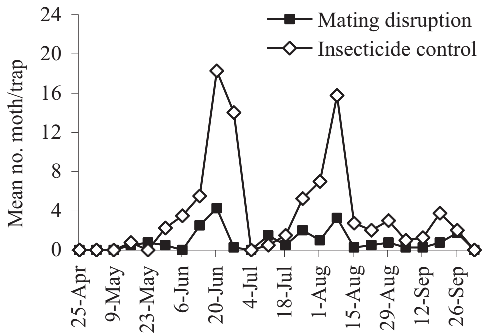
Insecticide applications for all mite and insect pests of cv. 'Gala' and 'Fuji' apples in mating disruption and insecticide plots in 2013 and 2014 are given in Table 1.

'Fuji' cultivar required 1.5 to 2 times more insecticide applications than cv. Gala as this variety was more susceptible to aphids, spider mites and codling moth (Yiem 1993; Joshi et al., 2015). When averaged over cultivars, mating disruption decreased the total number of sprays for apple pest complex by 40.70% and 56.60% in 2013 and 2014, respectively.

Depending on the years and cultivars, a total of four to 10 sprays was eliminated in the mating disruption plots. Insecticide applications for codling moth were reduced from three to six in the conventional programme to one in the mating disruption programme.



A) 2013



B) 2014

Figure 1. Mean codling moth catch in pheromone traps averaged across cultivars in mating disruption and insecticide plots in 2013 and 2014 in Bursa, Turkey

Depending on the years and cultivars, a total of four to 10 sprays was eliminated in the mating disruption plots. Insecticide applications for codling moth were reduced from three to six in the conventional programme to one in the mating disruption programme.

There were significant cost differences between conventional control and mating disruption in terms of materials, labour, and machinery (Table 2).

Table 1. Number of insecticide applications for all insect and mite pests of cvs. 'Gala' and 'Fuji' apples in mating disruption and insecticide plots in 2013 and 2014 in Bursa, Turkey

Variety	Insect pests[1]	2013			2014		
		Insecticide	Mating disruption	Difference	Insecticide	Mating disruption	Difference
'Gala'	Apple sawfly	1	1	0	2	2	0
	Aphids	2	1	1	2	1	1
	Budworm moths	0	0	0	0	0	0
	Codling moth	3	1	2	4	1	3
	San Jose scale	1	1	0	2	0	2
	Spider mites	2	1	1	2	1	1
	Total	9	5	4	12	5	7
'Fuji'	Apple sawfly	2	1	1	1	2	-1
	Aphids	4	3	1	5	3	2
	Budworm moths	1	1	0	1	0	1
	Codling moth	5	1	4	6	1	5
	San Jose scale	2	2	0	2	1	1
	Spider mites	4	3	1	3	1	2
	Total	18	11	7	18	8	10

[1]Apple sawfly = *Hoplocampa testudinea* (Klug); Aphids = *Dysaphis plantaginea* (Pass.), *D. devecta* (Walker), and *Eriosoma lanigerum* (Hausmann); Budworm moths = *Spilonota ocellana* (Den. & Schiff.) and *Hedya nubiferana* (Haworth); Codling moth = *Cydia pomonella* (L.); San Jose scale = *Quadraspidiotus perniciosus* (Comstock); Spider mites = *Panonychus ulmi* (Koch), *Tetranychus urticae* Koch, and *T. viennensis* Zacher

Table 2. Cost of materials, labour and machinery per ha compared between mating disruption and insecticide treatments consisting of cvs. 'Gala' and Fuji apples in 2013 and 2014 in Bursa, Turkey

Year	Cultivar	Treatment	Total no. applications[1]	Materials ($/ha)	Labour ($/ha)	Machinery ($/ha)	Total	Difference	Break-even analysis ($/dispenser)
2013	'Gala'	Insecticide	9	202.50	19.80	47.00	278.30		
	'Gala'	Mating disruption	6	420.00	31.00	15.00	472.00	-193.70	-0.19
	'Fuji'	Insecticide	18	382.50	39.60	150.00	590.10		
	'Fuji'	Mating disruption	12	532.50	44.20	61.00	649.70	-59.60	-0.06
2014	'Gala'	Insecticide	12	300.00	26.40	76.00	414.40		
	'Gala'	Mating disruption	6	368.00	31.00	19.00	424.00	-9.60	-0.01
	'Fuji'	Insecticide	18	397.50	39.60	170.00	625.10		
	'Fuji'	Mating disruption	9	442.50	37.60	40.00	529.10	96.00	*[2]

[1] Mating disruption treatments included one pheromone dispenser application plus insecticide applications.
[2] Since mating disruption was more cost-effective, there was no need to calculate a decrease in dispenser.

Mating disruption treatments lowered insecticide and machinery costs but increased labour costs compared with conventional treatments. Our findings are in agreement with those of Williamson et al. (1996).
Savings in insecticide expenditures varied from $ 120.00 in cv. 'Gala' to $ 255.00 in cv. 'Fuji'. Inconsistent with our results, Elkins et al. (2005) reported an average savings of $ 99 per ha in pesticide costs per year in pear orchards treated with pheromone puffers for control of codling moth. However, material costs in mating disruption plots were higher than insecticide plots due to the high cost of pheromone dispensers. The time-consuming installation procedure of pheromone dispensers caused a slight increase in labour costs in mating disruption blocks in most cases except for cv. 'Fuji' in 2014. Therefore, the cost of mating disruption ranged from $ 193.70 higher than the conventional treatment in 'Gala' in 2013 to $ 96.00 less than the conventional treatment in 'Fuji' in 2014. Similar to our cost calculations with cv. 'Gala' in 2013, Williamson et al. (1996) found in 'Red Delicious' apple orchards that codling moth mating disruption was $ 188.22 per ha more costly than conventional control on average. The cost difference could be perceived as the price of switching from conventional chemical control to mating disruption. Apparently,

management costs change from crop to crop, cultivar to cultivar, and year to year. Brumfield et al. (2004) demonstrated a lower of cost mating disruption for Oriental fruit moth, *Grapholita molesta* (Busck), in peach orchards containing cv. 'Redhaven', 'John Boy' and 'Encore', but not in those with cv. 'Bounty'. However, it is important to note that the researchers did not calculate machinery costs and labour in their study. Codling moth damage and cullage data were analyzed to compare the cost differences between the two treatments. The percent total culls in 2013 were 27.40% and 30.80% in insecticide and mating disruption plots, respectively, while they increased to 35.10% and 36.00% in 2014 in the same order. In apples, codling moth is responsible for about 10% of cullage, with an average of 2-3% damage (Hansen and Schievelbein, 2002). In fact, levels of percentage damage by codling moth larvae in traditional insecticide plots were 0.90% and 1.50% in 2013 and 2014, respectively (Table 3). On the other hand, percent larval damage was higher than the economic threshold of 2% for codling moth (Kovanci et al., 2010) in mating disruption plots with 2.20% and 3.70% infestation recorded on apples in 2013 and 2014, respectively. Our results showed that mating disruption did not cause any significant increase in cullage.

Table 3. Mean percent fruit damage by codling moth larvae averaged over cultivars in mating disruption and insecticide plots in Bursa, Turkey in 2013 and 2014.

		Damage[b](%)			
Year	Treatment	Sting	Entry	Larvae	Total
2013	Mating disruption	0.5 a	0.9 a	0.8 a	2.2 a
	Insecticide	0.5 a	0.3 a	0.1 a	0.9 a
2014	Mating disruption	1.5 a	1.1 a	1.1 a	3.7 a
	Insecticide	0.8 a	0.4 a	0.3 a	1.5 a

A break-even analysis was made based on the difference in costs between mating disruption and insecticide applications. To determine a break-even situation, cost differences for each cultivar and year were divided by the number of pheromone dispensers applied (1000/ha). Our findings indicated that about 22.22% price decrease in pheromone dispensers was needed to convince growers to use mating disruption in cv. 'Gala' apples in 2013. Even higher price reduction of up to 70.37% was necessary to achieve a break-even situation in cv. 'Fuji' apples in the same year. These results confirm the previous break-even analysis by Williamson et al. (1996), who suggested a 30-73% decrease in pheromone dispenser prices to 'Red Delicious' apple growers. To promote mating disruption, Turkish government offer subsidies of $ 125 per ha to encourage growers. However, there was no need for decrease in pheromone dispenser prices in 2014 because mating disruption programme was as cost-effective as insecticide programme.

This favourable change in costs of the two management programmes between years may have been caused by an increase in beneficial insects in apple orchards (Calkins, 1996). Evidently, the aphidophagous seven-spotted lady beetle, *Coccinella septempunctata* L., and *Typhlodromus athiasae* Porath and Swirski, the important predator of the European red mite, were more abundant in pheromone-treated orchards. In contrast, mating disruption may increase the risk of damage by some pests such as apple sawfly, *Hoplocampa testudinea* (Klug), which was previously suppressed by cover sprays. For example, two sprays were applied to control this pest in cv. 'Fuji' apples in mating disruption plots in 2014, while only one spray was applied in insecticide plots.

CONCLUSIONS

Based on partial budgeting analysis, mating disruption treatments lowered insecticide and machinery costs but increased labour costs compared with conventional treatments.

Codling moth mating disruption reduced the overall amount of insecticides used for managing apple pest complex. Despite these savings, the cost of mating disruption for codling moth averaged about $ 126.55 more than traditional insecticide applications in apple orchards in 2013.

A break-even analysis for the same year showed that a mean price decrease of 46.30% for pheromone dispensers would be required to convince growers to use mating disruption.

However, on average, mating disruption programme cost $ 43.20 less than a conventional insecticide programme in 2014.

Cost differences between years could be explained by varying codling moth populations, and different number of insecticide sprays.

The decrease in initial pest density, accompanied by enhanced biological control, may help us to develop more cost-effective mating disruption programmes in subsequent years.

ACKNOWLEDGEMENTS

The author is grateful for the travel grant by the Commission of Scientific Research Projects of Uludag University, Turkey.

REFERENCES

Brumfield R.G., Martin L.S., Polk D., Hamilton G. (2004). Costs of conventional versus low input peach production in the Eastern United States. Acta Horticulturae, 638:473-478.

Calkins C.O., 1996. Areawide IPM as tool for the future. In: Lynch S., Greene C., Kramer-LeBlance C. (Eds.), Proceedings of the 3rd National IPM Symposium / Workshop: Broadening support for 21st Century IPM. USDA, CA, USA, Mis. Pub. no. 1542:154-158.

Cardé R.T., Minsk, A.K., 1995. Control of moth pests by mating disruption: successes and constraints. Annual Review of Entomology, 40:559-585

Elkins, R.B., Shorey, H.H., 1998. Mating disruption of codling moth (*Cydia pomonella)* using “puffers”. Acta Horticulturae, 475:503-512.

Elkins R.B., Klonsky K.M., DeMoura R.L., 2005. Cost of production for transitioning from conventional codling moth control to aerosol-released mating disruption ("puffers") in pears. Acta Horticulturae (ISHS), 671:559-563.

Joshi N.K., Rajotte E.G., Myers C.T., Krawczyk G., Hull L.A., 2015. Development of a susceptibility index of apple cultivars for codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) oviposition. Frontiers Plant Science, 2015, 6: 992.

Hansen J.D., Schievelbein S., 2002. Apple sampling in packing house support the systems approach for export quarantine. Southwestern Entomologist, 27:277–282.

Kovanci O.B., Walgenbach J.F., Kennedy G.G., 2004. Evaluation of extended-season mating disruption of the Oriental fruit moth *Grapholita molesta* (Busck) (Lep., Tortricidae) in apples. Journal of Applied Entomology, 128:664-669. Kovanci O. B., Kumral N.A., Larsen T.E., 2010. High versus ultra-low volume spraying of a microencapsulated pheromone formulation for codling moth control in two apple cultivars. International Journal of Pest Management, 56:1-7.

Kovanci O.B., 2015. Co-application of microcapsulated pear ester and codlemone for mating disruption of *Cydia pomonella*. Journal of Pest Science, 88: 311-319.

Reyes M., Franck P., Charmillot P-J., Ioriatti C., Olivares J., Pasqualini E., Sauphanor B., 2007. Diversity of insecticide resistance mechanisms and spectrum in European populations of the codling moth, *Cydia pomonella*. Pest Management Science, 63:890-902.

Williamson E.R., Folwell R.J., Knight A.L., Howell J.F., 1996. Economics of employing pheromones for mating disruption of the codling moth, *Carpocapsa pomonella*. Crop Protection, 15:473-477.

Witzgall P., Stelinski L., Gut L., Thomson D., 2008 Codling moth management and chemical ecology. Annual Review of Entomology. 53:503-522.

Yiem M.S., 1993. Antixenosis and antibiosis of apple cultivars, Fuji, Starkrimson, Horei and Golden Delicious against *Tetranychus urticae* Koch. RDA Journal of Agricultural Science, Crop Protection 35, 409-413.

Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

NEW INVASIVE INSECT PESTS RECENTLY REPORTED IN SOUTHERN ROMANIA

Constantina CHIRECEANU, Andrei TEODORU, Andrei CHIRILOAIE

Research and Development Institute for Plant Protection Bucharest,
8 Ion Ionescu de la Brad, District 1, 013813 Bucharest, Romania

Corresponding author email: cchireceanu@yahoo.com

Abstract

This work presents the results of the field survey in 2016 referring to invasive insect pests newly recorded from the Southern Romania. Five non-European insect species, belonging to Hemiptera and Lepidoptera Orders damaging diverse plants were identified, as follows: three polyphagous species in Auchenorrhyncha group, Orientus ishidae, Phlogotettix cyclops and Acanalonia conica; one true bug species Leptoglossus occidentalis harmful to conifer seeds, and one leaf miner species Phyllocnistis vitegenella pest to grapevine. In this work we included summarized data related to some aspects of species origins, distribution in Europe and Romania and preference to the host plants.

Key words*: invasive species, insect pests, Southern Romania*.

INTRODUCTION

Phytophagous insects form a very important category of invasive species with a great rate of penetration in European territory. They are well represented by a wide spectrum of species belonging to numerous different taxonomic groups. According to Roques (2016), since 2000s the number of new phytophages that enter Europe is about 11.5 species per year. The same author reported that most of the exotic phytophagous species that established in Europe by 2014 are associated with woody plants, out of which insects represent 83.5%. Unintentionally introduced species have a greater velocity of spreading comparative to those deliberately introduced (Roques et al., 2016). Global costs associated with invasive insects reached a minimum of US $ 70.0 billion per year, and costs related to the health sector exceeded US $ 6.9 billion per year (Bradshaw et al., 2016).

Favored by natural factors (i.e. global warming, food chain) as well as intense human activities (i.e. commercial exchange, travel, tourism), the alien invasive species penetrate at a rapid pace from year to year in Romania as in other countries around the globe. In an attempt to detect the new exotic invasive species that entered Romania as soon as possible, and put together new accumulated data for a better understanding of the species distribution and richness, many research communities in institutions from different parts of the country were involved in issues related to invasive species.

The purpose of our research was to collect, identify and disseminate useful information on the new allochthonous invasive insect pests recorded in the Southern Romania in 2016, in order to bring an essential contribution to improve the available knowledge on this field to the country. Within our work, five invasive insect species, new for South part of Romania were detected, two of them being considered as the first report for Romanian pest fauna.

MATERIALS AND METHODS

The collecting of insects was performed in 2016 within the framework of the fields' survey program conducted by the Research-Development Institute for Plant Protection Bucharest in order to discover untimely pest insects having the status of invasive species. The sampling in 2016 was carried out in urban areas in Bucharest and rural areas in Ilfov and Giurgiu counties from the South part of Romania. Insects were captured with yellow sticky traps and by direct collection. To identify the insects in our samples we used the morphological features and illustrations describing adult specimens in relevant references in the literature. Using an Olympus camera connected to a TSZ 61 stereomicroscope we were able to take pictures of collected insects.

Geographical coordinate data for the sampling points were taken using the application Convertor online coordinate GPS: (http://www.calculatoare.ha-ha.ro/convertor_coordonate_gps_adresa.php; WGS84).

RESULTS AND DISCUSSIONS

The phytophagous insect species classified as invasive species, alien to Romania, that we encountered in the Southern Romania, during the vegetative season in 2016, are presented in Table 1.

Orientus ishidae (Matsumura, 1902) (Hemiptera: Cicadomorpha, Cicadellidae, Deltocephalinae) - the mosaic leafhopper species (Figure 1);

This species has East Asian origins. It has been recorded in the Northern Italy since 1998 (Guglielmino, 2005), and after that it rapidly spread to other European countries (EPPO, 2015).

In Romania, the first adults of *O. ishidae* have been described from the South part of the country (Bucharest) in 2016, by Chireceanu et al. (2017).

Table 1. Taxonomic position of invasive insect species detected in the Southern Romania in 2016; plant species on which the insects were found or traps were placed

Order	Family	Species	Common name	Host plants
Hemiptera	Cicadellidae	*Orientus ishidae* (Matsumura, 1902)	The mosaic leafhopper	Woody plants (*Crataegus monogyna*, *Malus domestica*, *Prunus avium*, *Ziziphus jujube*)
		Phlogotettix cyclops (Mulsant & Rey, 1855)		Woody plants (*Crataegus monogyna*, *Ziziphus jujube*, *Juglans regia*)
	Acanaloniidae	*Acanalonia conica* (Say, 1830)	The green cone-headed planthopper	Woody plants (*Crataegus monogyna*, *Malus domestica*)
	Coreidae	*Leptoglossus occidentalis* (Heidemann, 1910)	The western conifer seed bug	Building balcony
Lepidoptera	Gracillariidae	*Phyllocnistis vitegenella* (Clemens, 1859)	Grape leaf miner	Wild grapevine Hybrid *Vitis* sp.

Figure 1. Adult of *Orientus ishidae*

A number of 63 adult specimens were trapped in the course of the year 2016, on yellow sticky traps settled on apple (*Malus sylvestris*), sweet cherry *(Prunus avium),* Chinese date (*Ziziphus jujube*) and common hawthorn (*Crataegus monogyna*) trees present in urban areas of Bucharest.

The species of *M. sylvestris* (N44°30'05"/E26°4'35") and *P. avium* (N44°30'02"/E26°4'37") composed two research orchards of the Research-Development Station for Fruit Growing (RDSFG) in the Northern part of Bucharest; *Z. jujube* trees were enclosed in the experimental field of the USAMV Bucharest in the Northern part of Bucharest (N44°28'11"/E26°04'12") and in the 'D. Brândză' botanical garden of Bucharest in the central part of the capital (N44°26'17"/E26°03'52"); the shrubs of *C. monogyna* were present in a non-managed area (N44°30'15"/E26°04'02") near to the Research-Development Institute for Plant Protection and in the botanical garden of the USAMV Bucharest (N44°26'17"/ E26°03'52"), both institutions being situated in the North part of Bucharest.

O. ishidae is a polyphagous pest that lives on various plant species from woody plants and deciduous trees groups and feeds on the plant phloem. By reason of its strategy for feeding, the *O. ishidae* leafhopper is considered of great economic importance, because this was found to be associated to the spreading of some phytopathogenic microorganisms such as phytoplasmas (pathogens that live as obligate parasites in the phloem sieve tubes of plants and in insect vectors) to grapevine. The insect is a common presence in the vineyards in countries from West and Central Europe. Adults of O. *ishidae* collected in grapevine affected by yellows - type diseases, have been found infected with pathogens of the disease *Grapevine flavescence dorée* (GFD) (Mehle et al., 2011; Trivellone et al., 2015). Recent studies conducted in Italy (Lessio et al., 2016) have shown that *O. ishidae* was competent to transmit the GFD phytoplasma to grapevine. As regards the risk of FD to be spread by *O. ishidae* to grapevine, is believed to be real. This is because, in many references in the literature, *O. ishidae* is compared with *Scaphoideus titanus*, the main natural vector known to spread the GFD disease. Both species are Deltocephalinae leafhoppers belonging to the same family of Cicadellidae, and it is expected that *O. ishidae* to have the model of life cycle and behavior similar to *S. titanus*.

Phlogotettix cyclops **(**Mulsant & Rey, 1855) **(**Hemiptera: Cicadomorpha, Cicadellidae, Deltocephalinae); Syn. *Jassus cyclops* Mulsant & Rey, 1855 (Figure 2).

Figure 2. Adult of *Phlogotettix cyclops*

This leafhopper species is originally from Asian and Russian regions, spread in countries from Central and South East Europe on various plant species such as raspberry, fruit trees and grapevine (Chuche et al., 2010), this being known as a polyphagous species. The leafhopper has one generation per year and overwinters as eggs laid into plant tissues. First knowledge with regard to description of the presence of *P. cyclops* on grapevine was provided by Chuche et al. (2010) on Bordeaux vineyards. Like majority of the species in the Cicadellidae family, *P. cyclops* leafhopper is specialized in feeding on the phloem tissue of plants. From this reason, many scientists suspected this species as a possible new vector that may spread the phytoplasma pathogens to cultivated plants. The fruit trees and grapevine, crops of high economic importance, are mainly considered vulnerable to the risk of this insect. Wuu-Yang Chen et al. (2011) in East Asia has diagnosed the *P. cyclops* species as a serious vector with potential to transmit a strain of phytoplasma belonging to the 16SrI group. Reisenzein (2015) in Austria has found this cicadellid to be infected with phytoplasma of the *Flavescence dorée* disease, a grave systemic disease of grapevine in Europe. *P. cyclops* is cited in many reports in the European literature as present in Romania, based on the reports of Dlabola from 1977 and 1981 (www.faunaeur.org). After these reports, no article has been published on this species in Romania so far. In our field survey, 170 specimens of *P. cyclops* have been caught on yellow sticky traps settled on shrubs of common hawthorn (*Crataegus monogyna*) present in the area situated near the Plant Protection Institute (Northern Bucharest) (N44°30'15"/E26°04'02"), on trees of Chinese date (*Ziziphus jujube*) (N44°26'17"/E26°03'52") planted in the national Botanical Garden 'D. Branza' (Central part of Bucharest) and in the walnut (*Juglans regia*) orchard (N44°30'12"/ E26°15'49") in the Didactic Farm of USAMV Bucharest in Ilfov County at 15 km away from Bucharest.

Acanalonia conica (Say 1830) (Hemiptera: Fulgoromorpha, Acanaloniidae) - the green cone-headed planthopper (Figure 3).

This species that originates from North America was found for the first time in Europe in 2002, in Switzerland (Günther and Mühlethaler, 2002). In our field survey conducted in 2016, the adults of *A. conica* captured on yellow sticky traps accounted 21 specimens. The yellow sticky traps were placed on trees of common hawthorn occurring in the non-administered area (N44°30'15"/E26°04'02") around the court of the Plant Protection Institute and on an apple orchard (N44°30'05"/E26°04'35") that belongs to the experimental field of the Research Station in Pomiculture. Both sampling locations are situated in the North part of Bucharest.Within the captures of *A. conica* during this survey activity, we revealed for the first time the presence of this species in the Bucharest zone and in Romania as well (Chireceanu et al., 2017). In the examination of the traps, we noticed that the adults of *A. conica* captured were associated with those of another invasive planthopper species, the flatid *Metcalfa pruinosa*. *A. conica* is a univoltine species and overwinters as egg stage. Adults are bright green in color and measure 10 mm long. A typical feature of this species is the conical vertex of the head; they can be found during June-September (Aldini et al., 2008).

Figure 3. Adult of *Acanalonia conica*

In many reports in the European literature, *A. conica* is not considered a severe pest of cultivated plants because this generally develops small populations that are not able to produce essential damages. However, the grapevine and ornamental plants are indicated among the host plants with economic importance that are affected by this insect (D'Urso and Uliana, 2006).

Phyllocnistis vitegenella **(**Clemens, 1859) (Lepidoptera: Gracillariidae) - grape leaf miner (Figure 4b).

This is a North American species, reported in Europe for the first time in 1995, in the north-east of Italy (Cara and Jermini, 2011). Then, it has also been confirmed in Slovenia (2004), Switzerland (2009) and Hungary (2014) (Cara and Jermini, 2011; Szabóky and Takács, 2014). Adult of 3 mm, is distinguished by a brilliant white color with characteristic brown stripes very finely and two black dots on the apex of the wing. Larva produces visible injuries on leaves consisting in distinctive galleries (mines), very long and sinuous on the upper side of the leaves of plants belonging to Vitaceae family (Figure 4a). The average number of mines per leaf is maximum 4 (Lips and Jermini, 2013). The micromoth miner has 3-5 generations per year and spends the winter as adult.

For Romania, the first detection of the pest was on grapevine in Moldova region in 2013 (Ureche, 2016).

Research conducted in Switzerland focused on the grape leaf miner behavior, showed its preference for leaves of the shoots laterally disposed on vine plants. At the *Merlot* cultivar, the leaves of lateral shoots were mined over 3 times more than those of main shots (Lips and Jermini, 2013). The same authors regarded *P. vitegenella* as a minor pest of grapevine because this did not induce considerable negative effects and accordingly the control measures against its population are not necessary.

In our study conducted in the Southern Romania in 2016, we obtained *P. vitegenella* adults from mined leaves sampled from the grapevine rootstocks in a field previously planted with vine plants for research proposes within framework of the RIDPP Bucharest, and also from mined leaves sampled from two plots of hybrid grapevines in house gardens (Naipu village, Giurgiu County). In addition, we gathered *P. vitegenella* adults from leaves with mines collected from wild vines found in spontaneous flora around some vineyards in Vrancea County, on which we investigated in 2016 with regard to the presence of the American grapevine leafhopper *Scaphoideus titanus*. We have not discovered any sign of the attack of *P. vitegenella* on vine plants in the noble vineyards that we have monitored.

From the samples collected in the south part of the country and maintained in laboratory conditions, some parasitoids (Figure 4c) belonging to the Eulophidae family have resulted.

European research focused on parasitoids of the grape leaf miner *P. vitegenella* have indicated a parasitic rate of the pest up to 33%.The parasitoids associated with this species are framed to Eulophidae and Ichneumonidae families.

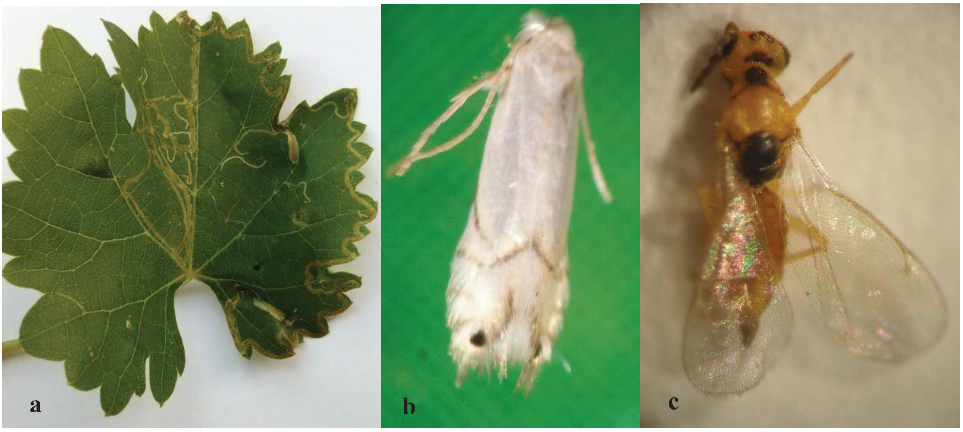


Figure 4. (a) Mined grapevine leaf; (b) Adult of *Phyllocnistis vitegenella*; (c) Parasitoid wasp from the Eulophidae family

Leptoglossus occidentalis **(**Heidemann, 1910) (Hemiptera: Heteroptera: Coreidae) - the western conifer seed bug (Figure 5).

L. occidentalis is a plant-feeding bug commonly called "leaf-footed bugs" or "squash bugs". It is a pest from North America, observed for the first time in Europe (Northern Italy) in 1999 (Taylor et al., 2001), from where this rapidly spread across the European continent, reaching up to Sweden and Ireland and even to Ukraine and Russia (Putchkov, 2013).

Figure 5. Adult of *Leptoglossus occidentalis*

It is a large true bug of approx 20 mm long (adults), easily recognizable by the hind legs strongly developed with a characteristic enlargement zone on the hind tibiae and long femurs serrated on the inside; thin visible white lines in the shape of inverted 'W' in the middle of the wings (Ruicănescu, 2009); the first segment of antennas is thicker, slightly curved, orange-brown with a black longitudinal line inwards. *L. occidentalis* has one or two generations annually depending on the altitude of area where it is living (Tamburini et al., 2012), and survives the winter conditions as adult, commonly in large groups, under coniferous trees bark, but also in people's homes and other buildings, from where they emerge in the spring season giving birth to the summer generation. It is a polyphagous insect on conifer species in the Pinaceae family (Tamburini et al., 2012). To date, the western seed bug has not been reported to produce important damage to conifers in Europe, but some overwintering adults of this species were detected to bear spores of the fungus *Diplodia pinea*, and from this point of view they are suspected to play a role in spreading of this pathogen (Tamburini et al., 2012). Instead, it is considered a nuisance pest to people, as the overwintering form enters into people's homes and walk unhindered on the walls (Rabitsch, 2008). In the Romanian territory, the presence of *L. occidentalis* (two females) was for the first time reported in the central part of the country, in 2008 and 2009 by Ruicănescu (2009). In the following years, the seed bug was reported in several other Romanian regions, in the Southeastern (Șerban, 2011), the Northwestern (Rădac and Petrovich, 2016), the Northeastern, Central and Southern parts (Olenici and Duduman, 2016) of the country. The pest had a low density, and the collecting points have always been associated with the conifer trees present around.

In our research, *L. occidentalis* (one male) was collected on October 28th, 2016, by the first author of this paper, on geranium plants in the balcony of her bloc apartment in the Southern area of Bucharest (44°23′34″/ 26°6′42″).

The presence of *L. occidentalis* inside of buildings in urban areas does not appear to be unusual because all Romanian papers on this subject previously published indicated the cover spaces as common collecting places for this species.Our results in this study confirm the presence of *L. occidentalis* in the Southern Romania and it may be considered the second record of this pest for this area and the first record for Bucharest so far. Capturing of the adults of the *L. occidentalis* bug during the October and December months is explained by the fact that the insects are seeking shelter for wintering (Olenici and Duduman, 2016).

CONCLUSIONS

The field survey and sample evaluation performed by us in areas from the Southern Romania in 2016, led to obtaining relevant knowledge on the presence of five non-European important insect species, belonging to the Hemiptera and Lepidoptera Orders, such as: three polyphagous species in Auchenorrhyncha group, *Orientus ishidae, Phlogotettix cyclops* and *Acanalonia conica;* one true bug species *Leptoglossus occidentalis* harmful to conifer seeds, and one leaf miner species *Phyllocnistis vitegenella* pest to grapevine.

The results obtained within this work suggest that the activities of the field survey are critical for the early detection of alien invasive species that could unexpectedly penetrate into new territories, unaffected by them until then. Further data on the spreading in other areas as well as the incidence on plants for the new detected invasive species will be essential for the Romanian territory in the near future, so that the surveys in the field would be extended to many other crops, mainly those of economic importance, such as fruit trees, grapevine, ornamentals and conifers.

ACKNOWLEDGEMENTS

The study was performed in the framework of the project PN 16-29-01-01, financial support by the Ministry of Research and Innovation.

REFERENCES

Aldini R. N., Mazzoni E., Mori N., Ciampitti M., 2008. On the distribution in Italy of the Nearctic hopper Acanalonia conica, with ecological notes. Bulletin of Insectology, 61(1):153-154.

Bradshaw C. J. A., Leroy B., Bellard C., Roiz D., Albert C., Fournier A., Barbet-Massin M., Salles J-M., Simard F., Courchamp F., 2016. Massive yet grossly underestimated global costs of invasive insects. Nature Communications 7, 12986, DOI:10.1038/ncomms12986.

Cara C., Jermini M., 2011. La mineuse américaine Phyllocnistis vitegenella, un nouveau ravageur de la vigne au Tessin. Revue suisse Viticulture, Arboriculture, Horticulture, 43:224-230.

Chireceanu C., Teodoru A., Gutue M., Dumitru M., Anastasiu P., 2017. Two new invasive hemipteran species first recorded in Romania: Orientus ishidae (Matsumura 1902) (Cicadellidae) and Acanalonia conica (Say 1830) (Acanaloniidae). Journal of Entomology and Zoology Studies, 5(2): 824-830

Chuche J., Danet J.-Luc., Thiery D., 2010. First description of the occurrence of the leafhopper Phlogotettix cyclops in a Bordeaux vineyard. Journal International des Sciences de la Vigne et du Vin, 44:161-165.

Dlabola J., 1977. Chorologische Ergünzungen zur Zikadenfauna des Mittelmeergebdetes (Horn.: Auchenorrhyncha). Acta Entomologica Musei Nationalis Pragae, 33(1- 21):21- 31.

Dlabola J., 1981. Eregbnisse der Tschehoslowaıkisch Iranisohen Entomologischen Expeditionen nach dem Iran (1970 und 1973) (Mit Augaben über einige Salmelresultate in Anatolienl Homoptera: Auchenorrhyncha m. Ten). Acta Entomologica Musei Nationalis Pragae, 40:127- 311.

D'Urso V., Uliana M., 2006. Acanalonia conica (Hemiptera, Fulgoromorpha, Acanaloniidae), a Nearctic species recently introduced in Europe. Deutsche entomologische Zeitschrift, 53(1):103-107.

Günthart H., Mühlethaler R., 2002. Provisorische Checklist der Zikaden der Schweiz (Insecta: Hemiptera, Auchenorrhyncha). Denisia, 4(176):329-338.

Lessio F., Picciau L., Gonella E., Andrioli M., Tota F., Alma A., 2016. The mosaic leafhopper Orientus ishidae: host plants, spatial distribution, infectivity, and transmission of 16SrV phytoplasmas to vines. Bulletin of Insectology, 69(2):277-289.

Lips A., Jermini M., 2013. Harmfulness of the American grape leaf miner Phyllocnistis vitegenella on the grapevine ‘Merlot’ (Vitis vinifera). Federal Department of Economic Affairs, Education and Research EAER, Agroscope.

Mehle N., Rupar M., Seljak G., Ravnikar M., Dermastia M., 2011. Molecular diversity of 'flavescence dorée' phytoplasma strains in Slovenia. Bulletin of Insectology, 64(suppl.): S29-S30.

Olenici N., Duduman M. L., 2016. Noi semnalări ale unor specii de insecte forestiere invazive în România. Bucovina Forestieră, 16(2), 161-174.

Putchkov P. V., 2013. Invasive true bugs (Heteroptera) established in Europe. Український ентомологічний журнал, 2(7):11-28.

Rabitsch W., 2008. Alien True Bugs of Europe (Insecta: Hemiptera: Heteroptera). Zootaxa 1827:1-44,

Rădac I. A., Petrovici M., 2016. Studies regarding the true bugs fauna (Insecta, Heteroptera) in Cefa Nature Park. Acta Oecologica Carpatica, 9:121-132.

Reisenzein H., 2015. Epidemiological studies on flavescence dorée (FD) IOBC meeting, Vienna, 1-27. http://www.faunaeur.org/distribution_table.php (last update 29 August 2013; 23.01.2017 visited).

Roques A., Auger-Rozenberg M. A., Blackburn T. M., Garnas J., Pyšek P., Rabitsch W., Richardson D. M., Wingfield M. J., Liebhold A. M., Duncan R. P., 2016. Temporal and interspecific variation in rates of spread for insect species invading Europe during the last 200 years. Biol Invasions, 18:907-920.

Ruicănescu A., 2009. Leptoglossus occidentalis Heidemann, 1910 (Heteroptera: Coreidae) in Romania. In Rakosy L., Momeu L. (Eds.), Neobiota in România. Presa Universitară Clujeană, Cluj-Napoca, 153-154.

Şerban C., 2011. Leptoglossus occidentalis Heidemann, 1910 (Heteroptera: Coreoidea): a new record for the invasive true bugs fauna of Romania. In Murariu D.C., Adam G., Chişamera E., Iorgu L. O., Popa O. P. (Eds.), Annual Zoological Congress of “Grigore Antipa” Museum. Book of abstracts, “Grigore Antipa” National Museum of Natural History, Bucharest, Romania, 102.

Szabóky C., Takács A., 2014. The first occurrence of American grape leaf miner (Phyllocnistis vitegenella Clemens, 1859-Gracillariidae) on grapevine (Vitis vinifera) in Hungary. Növényvédelem, 50(10):467-469.

Tamburini M., Maresi G., Salvadori C., Battisti A., Zottele F., Pedrazzoli F., 2012. Adaptation of the invasive western conifer seed bug Leptoglossus occidentalis Trentino, an alpine region (Italy). Bulletin of Insectology, 65(2):161-170.

Taylor S. J., Tescari G., Villa M., 2001. A Nearctic pest of Pinaceae accidentally introduced into Europe: Leptoglossus occidentalis (Heteroptera: Coreidae) in northern Italy. Entomological News, 112(2):101-103.

Trivellone V., Filippin L., Jermini M., Angelini E., 2015. Molecular characterization of phytoplasma strains in leafhoppers inhabiting the vineyard agroecosystem in Southern Switzerland. Phytopathogenic Mollicutes, 5 (suppl.):S45-S46.

Ureche C., 2016. First record of a new alien invasive species in Romania: Phyllocnistis vitegenella Clemens (Lepidoptera: Gracillariidae). Acta Oecologica Carpatica, IX: 133-138.

Chen WY., Huang YC., Tsai ML., Lin CP., 2011. Detection and identification of a new phytoplasma associated with periwinkle leaf yellowing disease in Taiwan. Australasian Plant Pathology 40: 476. DOI 10.1007/s13313-011-0062-x.

EPPO Reporting Service no. 05-2015 Num. article: 2015/098. Orientus ishidae: a potential phytoplasma vector spreading in the EPPO region.