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EFFECT OF THE MAIN CYTOKININS ON ANDROGENESIS OF WHITE CABBAGE (*BRASSICA OLERACEA* L. var. *CAPITATA*) ANTHERS CULTIVATED "IN VITRO"

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ABSTRACT

The aim of the present research work was the screening of the effect of the main cytokinin (BAP, kinetin or zeatin) in different concentrations and combinations with the auxin NAA on androgenesis of white cabbage anthers cultivated *in vitro.* The results obtained are regarded as an intermediary stage for the development of a reproducible protocol for *in vitro* regeneration of plant from anther culture.
Thus, for the determination of the influence of plant growth regulators formula over the callus induction and plant regeneration from anthers cultivated *in vitro* in the present study the authors undergo a screening of the three most frequently utilized cytokinins (BAP, kinetin and zeatin) in different concentration and combination with the auxin NAA. The results obtained, indicated that the best morphogenetic reaction is obtained on variant with BAP as the main growth regulator.

Keywords: benzylaminopurine, kinetin, zeatin, haploid, morphogenesis

INTRODUCTION

The *Brassica* group is a large group of plants which contains many commercially important food and oilseed crop plants. The vegetable crops from this group, such as cabbage, cauliflower, broccoli and brussels sprouts belong to *B. oleracea* and they are essential for human nutrition due to their high content in vitamins and minerals being cultivated on large surfaces all over the world (Favela-González et al., 2020; Murat et al., 2018).
In *Brassica* spp. breeding programs, *in vitro* plant tissue cultures has been involved in a wide range of applications. Among these, haploid production through anther culture proved to be an important approach of tissue culture, during the last decades (Shariatpanahi and Ahmadi, 2016; Anshul et al., 2020). If traditionally, breeders can obtain homozygosity by self-pollination, in 8-10 years, with anther culture, homozygous plant can be produced within one year. Theanther culture is an alternative and efficient technique to conventional breeding methods by enabling production of haploid and di-haploid plants. This is the reason why the scientific communities show a huge interest in improvement the existing *in vitro* anther culture techniques in different *Brassica* species including *B. napus*, *B oleracea*, *B. campestris*, *B. juncea*, *B. carinata* and *B. nigra.* Due to the rather recalcitrant reaction of *Brassica* tissues to *in vitro* culture, the researchers are focused on the establishment of the most effective conditions that allow the development of embryos, shoots and finally plants from anthers culture in order to obtain microspore derived haploids (MDH). The regeneration of haploid plants depends on a wide range of factors, from which important are: genotype, plant growth regulators (PGR), culture media, physiological status of donor

plant, stage of pollen development, temperature and light (Fowler, 2000; Vishal, 2012; Zhang, 2003; Monisha and Saikat, 2020).
Plant biotechnology has been used for decades as a tool to improve the plant breeding programs. Among the many techniques employed, anther culture is designed for generating haploid plants which through different diploididization methods can be transformed in homozygous dihaploids, utilizable as parental lines for F1 hybrids. Plant breeders, traditionally, achieve homozygosity of the cross products through self-fertilization - which usually needs 8-10 year, by anther culture, homozygous plant can be produced within a year. Many factors influence the morphogenetic reaction of anthers cultivated on solid media *in vitro.* Among them, the most important are: the plant growth regulator added in culture media, genotype physiological status of donor plant, anther wall factor, stage of microspore development, and effect of temperature and light. All these inner and outer factors basically influence the future evolution *in vitro* of anthers either toward callus induction or plant regeneration directly from immature anther. The importance of plant growth regulators added to the *in vitro* culture medium for callus induction, organ formation and embryogenesis, has been demonstrated for a large number of plant species. Among the protocols in which cytokinins were used as PGR for induction of regeneration, the literature shows that N6-benzylaminopurine (BAP) was the most frequently employed (57%), followed by kinetin (37%), zeatin (3%) and thidiazuron (3%).

MATERIALS AND METHODS

Plant material
The biologic material was represented by unopened flower buds at 3.0 – 3.4 mm length, which contain anthers with microspores at late uninucleate or binucleate stage. The developmental stage of the microspores was established through observation under microscope according with 1% aceto-carmine method. The buds were collected from mother plants belonging to the variety – DL20 developed and maintained at Vegetable Research and Development Station Bacau. The mother plants were grown in controlled conditions in greenhouse, with proper regime of fertilisation, irrigation and phyto-sanitary control.
Sterilization
The excised buds were surface sterilized in 0.1% mercuric chloride (w/v) for 15 min, followed by rinsing in sterile distilled water for 3 to 4 times. After sterilization the buds were dissected under aseptic conditions, the anther filaments removed and the anthers were inoculated in sterile tubes, on NLN (Lichter, 1982) basal nutrient medium (macroelements, microelements and vitamins)containing 3% (w/v) sucrose,50μM $AgNO_3$ and supplemented with different quantities of cytokinin (BAP, Kinetin or zeatin) and auxin NAA – table 1.
The pH was adjusted to 5.8 prior to the addition of 0.8% agar and autoclaved at 121°C (1.06 kg/cm^2) for 25 min.
Culture techniques
The cultures were incubated at 33°C temperature for one week in complete dark, followed by their transfer in culture chambers with controlled light (16-h photoperiod, and 5000 lx light intensity), and temperature at (25^0C). Four to five weeks after inoculation the anthers were removed aseptically from the culture tubes and were transferred on freshly prepared sterilized medium with the same PGRs formula.

Table 1. PGR combinations tested for their role on androgenetic potential of *Brassica*

Variant	BAP (µM)	KIN (µM)	ZEATIN (µM)	NAA (µM)
V_0	-	-	-	-
V_1	4.4	-	-	1.6
V_2	4.4	-	-	2.7
V_3	4.4	-	-	5.4
V_4	8.9	-	-	2.7
V_5	8.9	-	-	-
V_6	-	4.6	-	1.6
V_7	-	4.6	-	2.7
V_8	-	4.6	-	5.4
V_9	-	9.3	-	2.7
V_{10}	-	9.3	-	-
V_{11}	-	-	4.6	1.6
V_{12}	-	-	4.6	2.7
V_{13}	-	-	4.6	5.4
V_{14}	-	-	9.1	2.7
V_{15}	-	-	9.1	-

The sub-cultured culture tubes were then incubated at 25°C with 16 h photoperiod for 5-7 days. Repeated sub cultures were done at an interval of 30 days and incubated under the same temperature as mentioned previously. The culture vessels showing signs of contamination were discarded. Day to day observations were carried out to note the responses.

Rooting and acclimatization

After 3 to 4 weeks, when regenerated shoots reached a length of more than 4.0 cm, they were separated and transferred on NLN basal medium supplemented with 2.7 µM NAA for rooting. The rooted plantlets were transferred to the hydroponics conditions in bottles and hardened by maintaining a high humidity (90% RH) during first week. Then, by gradually decreasing the humidity, resulted a survival rate of over 95% of the plants.

Statistical analysis

Ten anthers per sterile tube, in three replications were inoculated for each variant. The percentage of anthers forming regenerative structures and the mean number of shoots per explant were recorded. The data were analyzed by ANOVA.

RESULTS AND DISCUSSIONS

Beside callusogenesis, the main morphogenetic responses of anthers on the 15 variants tested in the present study were organogenesis, and/or embryogenesis. Stereomicroscopic observations revealed that the regenerative structures got initiated after 14 - 20 days of cultures. The anthers started to grow in size and small protuberances emerged on the anther's surfaces that gradually evolved toward callus or direct regeneration of shoot and embryoids from anthers. In this stage the anthers acquired either directly or indirectly (which involved the callus phase) organogenic and embryogenic competence.

The poor regeneration response found on control variant (V0) lead us to the conclusion that the addition of PGRs in culture medium is essential for inducing morphogenetic competences in anthers' tissues (organogenesis, or embryogenesis) and afterwards plant regeneration. Among the PGRs tested, the best responses were found on variants with BAP.

The anthers cultivated on these media showed higher results both for indirect and direct organogenesis and embryogenesis- table 2. Similar results were obtained with culture media with zeatin, that induced either direct regeneration of somatic embryos and/or shoots from the tissues of the anthers, or indirect regeneration from calli with organogenic structures. The most effective medium variant was V4, containing 8.9 μM BAP and 2.7 μM NAA. On this variants 59,3% from reactive anther proved to have organogenic competences, 30,4% embryogenic competences, and only 10.2% differentiated calli. Due to the fact that is desirable to obtain the plants directly from the anthers, we considered this variant as being one of the most effective combinations of PGRs for plant regeneration.

Table 2. Effect of PGR on callus induction (callusogenesis), organogenesis, embryogenesis and plant regeneration in anther culture of *B. oleracea* (mean±SE)

Variant	organogenesis		embryogenesis		callusogenesis	
	%	Average no. of shoots/exp	%	Average no. of shoots/exp	%	Average no. of shoots/exp
V_0	15.9	4.69±0.18	19.1	0.41±0.46	65.0	5.62±0.24
V_1	35.9	11.52±0.32	28.3	1.70±0.38	35.7	14.17±0.41
V_2	38.6	14.78±0.36	22.1	2.02±0.28	39.2	18.03±0.29
V_3	18.7	16.66±0.52	29.6	2.13±0.52	51.6	19.80±0.64
V_4	59.3	21.33±2.21	30.4	3.18±0.60	10.2	25.51±0.98
V_5	33.7	16.09±0.48	21.6	1.42±0.29	44.5	19.79±0.14
V_6	18.5	7.33±0.35	7.8	0.38±0.34	72.8	8.20±0.31
V_7	10.3	7.28±0.28	0.4	0.62±0.12	89.1	8.51±0.45
V_8	26.7	6. 44±0.18	4.0	0.85±0.24	68.2	7.79±0.30
V_9	45.5	9.33±0.35	4.4	1.00±0.20	50.4	10.44±0.29
V_{10}	21.8	8.21±0.33	3.7	0.57±0.10	74.4	9.67±0.71
V_{11}	31.5	12.42±0.15	31.1	1.50±0.15	34.9	14.77±0.20
V_{12}	31.3	13.27±0.72	27.8	2.00±0.15	38.4	15.81±0.63
V_{13}	28.8	11.88±0.24	17.9	2.18±0.32	50.8	14.01±0.35
V_{14}	38.5	17.95±0.88	29.4	2.22±0.19	29.6	21.39±0.17
V_{15}	25.5	14.54±0.98	16.1	1.70±0.34	57.9	17.44±0.84

The effect of PGRs on the number of anthers that generated callus

Still, one of the most frequent regeneration responses was callus formation. On all media, including control medium, the anthers generated small calli, mainly on the filament side of the anthers in two three weeks after inoculation. Transferred calli on fresh media with the same composition of PGRs developed shoots and somatic embryos. Better results were noticed on media with BAP and zeatin as cytokinin. The stereomicroscopic observations underlined two types of callus: embryogenic and nonembryogenic. The embryogenic callus, with a relatively undifferentiated structure had isodiametric cells and areas with somatic embryos and shoots in different areas and in different stages of development, while the nonembryogenic callus had large, anisodiametric cells, with tracheides but no regenerative areas. The type and concentration of PGRs determined also differences in the colour of callus. Thus, on media with BAP and zeatin the predominant colour of callus varied between white green to dark green, while on media with kinetin the calluses were mainly white or white-brownish. The results obtained revealed that the consistence of callus obtained on media with kinetin was friable, non regenerative, the rate of regenerated shoots per callus being relatively low when compared with BAP or zeatin containing media.

Plant regeneration started two weeks after the transfer of calli to fresh media. Some of them regenerated both green and albino plantlets. Plant regeneration was greatly affected by the type of cytokinin added to medium. Thus, on media with kinetin the regeneration were significantly reduced, while BAP promoted the development of shoots from callus. The shoots, aseptically removed from tubes and placed on new fresh media allowed the continuation of regeneration processes through the apparition of adventitious shoots at the base of the newly formed ones.

The effect of PGRs on the number of anthers that generated shoots and embryos

The frequency of adventitious shoot regeneration was highly influenced by growth regulator concentration and combination. During the first week after inoculation, the first visible change in anthers was their slight enlargement in size. Adventitious shoots developed especially on the filament side of anthers and initially appeared as small multiple outgrowths. Regeneration percentage was affected by the addition of naphthalene acetic acid (NAA) with higher regeneration at higher concentrations (Table 2). Among the different combinations of plant growth regulators tested, NLN medium supplemented with BAP 8.9 µM and NAA 2.7 µM gave the best results, with higher frequency of direct shoot regeneration (59,3%) and number of shoots per explant (21.33 ± 2.21).

The embryos formation was also higher on this variant, 3.1±0.60 embryos per anther being the highest value obtained. At lower concentrations of BAP (4.4 µM) the frequency of embryo and shoot regeneration and the number of shoots per explant decreased.

The addition of zeatin in culture media also proved to be effective in Brassica anthers cultivated *in* vitroThe combination of zeatin with NAA was also beneficial for shoots and embryos development the values obtained on these variants being higher when compared with zeatin alone.

The kinetin proved to be less effective for inducing the shoot and embryo formation. On the variants with kinetin both, the frequency of shoot and embryo regeneration, and the number of embryos and shoots per anther were lower.

The induction of callus was the main morphogenetic reaction as 50.79% from total reactive anthers, gained callusogenetic competence. Direct organogenesis was observed at 30.03% of anthers, where small meristematic centers started to appear directly on the surface of globular-shaped anthers. Only 18.35% from total reactive anthers generated proembryo and embryo directly from anther's tissue.

After the first plantlets appeared, they were removed aseptically from the culture tubes and transferred on freshly prepared sterilized medium containing the same combination of PGRs. Gradually, at the basis of each new plantlet, both on the surface and inside the medium started to appear small meristematic centers that evolved in shoots. The transfer of the shoots on NLN basal medium supplemented with 2.7 µM NAA allowed root formation. Rooted plants were hardened by maintaining a high humidity (90% RH) during first week of hardening, which resulted in more than 80% survival of plantlets.

While Górecka et al. (1997)reported a better response on B5-2 medium, containing sucrose (20 g/l) and kinetin (20 mg/l), in the experimental conditions of our study the ability of anthers to gain organogenic and embryogenic competence was higher on NLN medium supplemented with BAP 8.9 µM and NAA 2.7 µM .

CONCLUSIONS

- The results highlighted in the present study show that the morphogenetic reaction of anthers is highly linked to the addition of PGRs in culture media, the type of PGRs and their quantities, as it directly influence the ability of anthers to gain organogenic and embryogenic competence. Thus, on Brassica anthers the most effective PGRs combination,

among the ones tested by us, was BAP 8.9 μM with NAA 2.7 μM with the highest frequency of direct shoot regeneration and number of shoots per explant.

- Shoot development and rooting were successful on NLN basal medium supplemented with 2.7 μM NAA, and acclimatized plants were transferred to grow to maturity in the greenhouse.

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IN VITRO MORPHOGENETIC REACTION OF *MELISSA OFFICINALIS* L.

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ABSTRACT

Lemon balm (*Melissa officinalis*L.) is a medicinal plant with a long history in traditional medicine. Classical propagation of this species is inefficient for establishing a good quality clonal plants. The aim of this work was to elaborate an *in vitro* propagation protocol for *M. officinalis* using apexes and uninodal fragments as explants. The highest multiplication rate (4.7 shoots/explant) was obtained on a MS medium supplemented with 3 mg/L BAP. A half strength MS medium supplemented with 1 mg/L NAA was the most effective for *in vitro* rooting of lemon balmmicroshoots. Micropropagated plants transferred *ex vitro* showed normal morphology and 95% survival rate during acclimatization. The results obtained throughout the *in vitro* regeneration phases confirm that *in vitro* tissue culture is an efficient method for multiplication of *M. officinalis*.

Keywords*:* lemon balm, micropropagation, growth regulators, multiplication, rooting

INTRODUCTION

Melissa officinalis L.(lemon balm) is a herbaceous, perennial plant of *Lamiaceae* family, native to northern Mediterranean region, known for the meliferous and curative properties (Tavares et al., 1996).The aerial part of plant comprises 0.05 to 0.15% of volatile oil (that contains citronellal, citral, geraniol, linalool), polyphenols, tannins (3 to 6%), mucilages (12%), bitter substances etc. The seeds contain fat oil made up of linolenic, linoleic, oleic, palmitic and stearic acids (Ciulei et al., 1993; Schultze et al., 1993; Parvu, 2000; Stanescu et al., 2002; Tita, 2003; Aprostosoaie, 2005). The main action of its active principles, especially of lemon balm volatile oil is spasmolitic, sedative, antiseptic, carminative, choleretic, mild laxative, stomachic, cicatrisant, galactagogue and insecticide (Ciulei et al., 1993; Stanescu et al., 2002; Tita, 2003).

M. officinalis is naturally propagated by seeds or vegetatively and grows easily in farm, but its population is not homozygote and the yield is extremely low. Therefore, its production via cultivation of local populations using traditional methods is not economic (Meftahizade et al., 2010a). The *in vitro* culture of aromatic and medicinal plants has proved to be an important alternative for rapid multiplication of selected genotypes (Agostini and Echeverrigaray, 2006). *In vitro* regenerated plants are often healthier than their field propagated clones, this is mainly due to rejuvenation and they are often disease-free plants (Pierik, 1997). Shoot proliferation from apices or axillary buds to produce multiple shoots with root production is now recognized as a viable technique for plant propagation (Tavares et al., 1996). Micropropagation is a valuable method for large scale multiplication of manyplant species, but the appropriate use, type and concentration of growth regulators

and the combination of culture medium salts that allows fast, efficient development of theinitial explants are crucial in tissue culture techniques (Schuchovski and Biasi, 2019). Several researchers work to standardize the optimum concentrations of growth regulators for shoot proliferation and regeneration of lemon balm (Schultze et al., 1993; Tavares et al., 1996; Meszaros, 1999; Da Silva et al., 2005; Ghiorghita et al., 2005; Meftahizade et al., 2010 a, b).
Considering the medicinal importance of *M. officinalis* we intended to find out some information regarding its *in vitro* behavior, the reaction of explants on varied hormonal formulae and the possibility of identifying an effective micropropagation technology.

MATERIALS AND METHODS

"In vitro" culture initiationphase

The explants used for the initiation of *in vitro* cultures consisted of apexes and uninodal fragments from actively growing shoots of *M. officinalis* mother stock plants. The shoots were first rinsed in tap water and were sterilized in 6% calcium hypochlorite solution for 10 minutes, followed bythree rinses using sterile distilled water.The stem segments were then cut with a sterile scalpel blade into smaller segments (1-1.5 cm long), each with one node used as explants. The explants were placed vertically on a plain MS medium (Murashige and Skoog, 1962) and maintained as shoot tip and single node cultures until plant material was sufficient for further experiments. The inoculation of explants was carried out under aseptic conditions using a laminar air flow hood.At this stage of the experiment, as well as at subsequent stages, the medium was supplemented with 40 g/L glucose, 32 mg/L NaFeEDTA (as iron source) and 7 g/L agar (for solidification of culture media).The culture media were sterilized by autoclaving at 120°C for 20 minutes. Before autoclaving, the pH of the medium was adjusted to 5.6-5.8 with 1N KOH or 1N HCl.All cultures were transferred in a growth room with controlled conditions at 22-24^{o}C, a 16 hourslight photoperiodat 3000 lx.

"In vitro" multiplication phase

Shoot proliferation was induced on a full strength MS medium supplemented with different type of cytokinins (BAP-benzylaminopurine, KIN-kinetin) at various concentrations (0,1, 1.5, 2 and 3 mg/L). Subculturing was performed every four weeks.The number of shoots per explant and shoot length was monitored as growth parameters. Every treatment was performed in three repetitions.

"In vitro" rooting phase

Individual microshoots were transferred in a half strength MS medium, supplemented with three different auxins (NAA-naphthalenacetic acid, IAA-indolylacetic acid, IBA-indolylbutyric acid) at concentration of 0, 0.5 and 1 mg/L. After four weeks of culturing, the rooting rate (ratio between the number of shoots at which the rhizogenesis process took place and the total number of shoots transferred to the rooting culture medium) was evaluated. Every treatment was performed in three repetitions.

Acclimatization phase

The *in vitro* rooted plantlets were removed from culture medium and their roots were washed in running tap water and then transplanted in pills of peat (Jiffy) for acclimatization to *ex vitro* conditions. Since lemon balm leaves are very sensitive to water loss and the loss of the water content of plantlets is irreversible, it was necessary to provide a high humidity environment by placing the plants under a plastic foil tunnel and spraying them with water until they start to harden.The percentage of acclimatized plants (the ratio between the number of viable plants and the total number of plants transferred *ex vitro*) was calculated after four weeks.The acclimatized plants were then transplanted in 0.5 L plastic pots for

fortification and maintained in a non-heated greenhouse for further growth and development.

Statistical analysis

The experimental design was planed in triplicates for each treatment.Statistical interpretation of the data was done using SPSS 10 for Windows program. Differences between variantscompared to the controlwere analyzed with One Way ANOVA – LSD, considering to be significant at $P < 0.05$.

RESULTS AND DISCUSSIONS

Morphogenetic reaction of explants in the initiation phase of"in vitro" culture

Culture initiation is the most important stageof micropropagation in different plant species. One of the essential conditions on which depends the success of initiation and maintenance of a cell culture is that of ensuring asepsis. The method of the biological material sterilization varies depending on the origin of the material, the physiological state and the type of explant material.In the present study, for *in vitro* culture initiation were used as explants apexes and uninodal fragmentssampled from actively growing shoots. Our observations highlighted the fact that including the lemon balm in this culture system does not pose particular problems, use of calcium hypochlorite (6% solution for 10 minutes) for biological material sterilization proved to be efficient.The use of a plain MS basal medium induced the successfulshoot development and microplantproduction, which were then used for experimentation of *M. officinalis* explants for morphogenetic reaction on different concentration of cytokinins (Figure 1).

Figure 1. *M. officinalis*microplants regenerated on plain MS medium,four weeks after *in vitro* cultureinitiation

Effect of cytokinintype and concentration on "in vitro"shoot proliferation

The current experimentswere carried out using 6-benzylaminopurine (BAP) and kinetin (KIN).BAP showed better response regarding the development of shoots than kinetin. Kinetin produced a lower number of shoots per explant and shoot length, compared to BAP treatments.The results obtained revealed that the maximum number of shoots per explant was obtained on MS medium supplemented with 3 mg/LBAP (4.7 shoots/explant)followed by the medium with 2 mg/LBAP (4.5 shoots/explant)(Figure 2). This is coinciding with the previous observations revealingthat the increase of BAP

concentration gave greatest efficiency in shoots number (Tavares et al., 1996; Sato et al., 2005). The high efficiency of BAP in shoot proliferation resulted in several medicinal plant species which are well documented in the literature (Mikulik, 1999; Rout et al., 2000; Fracaro and Echeverrigary, 2001; Kamstaityte and Stanys V., 2004; Rout, 2004; Balogun et al., 2007; Bohidar et al., 2008; Kalimuthu et al., 2010).

This fact can be explained that cytokinins, especially at high concentrations, overcome theapical dominance and inhibit the effect of apical peak on side buds, stimulating the proliferation of axillary shoots from these buds (Mikulik, 1999; Fracaro and Echeverrigary, 2001).

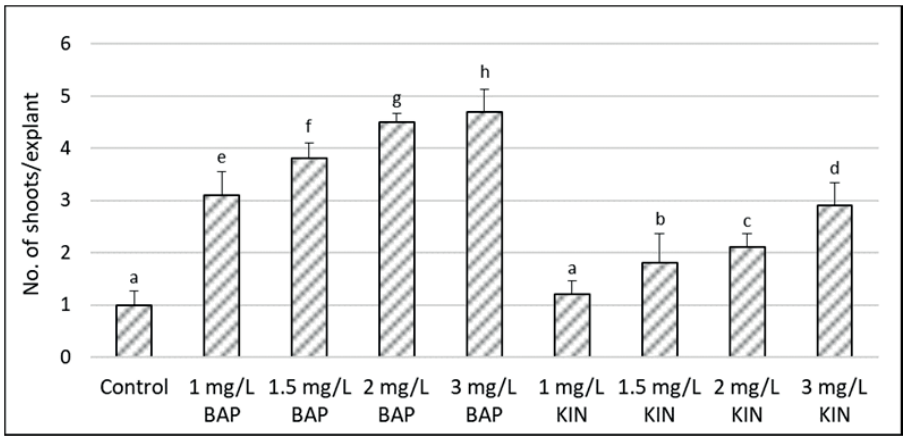

Figure 2. The influence of type and concentration of cytokinin on the number of shoots per explant of *M. officinalis*. The mean values are accompanied by their corresponding standarddeviations; letters indicate significance of differences as compared to the control at P <0.05

Tavares et al. (1996) also reported that higher concentration of BAP induced more but smaller shoots, suggesting an inverse relation between the number of shoots and their elongation. The treatment of 3 mg/L BAP induced the largest number and the longest shoots. These results are in concordance with the results obtained by Gulati and Jaiwal (1994) which reported at *M. officinalis* a direct relationship between the number of shoots and their elongation

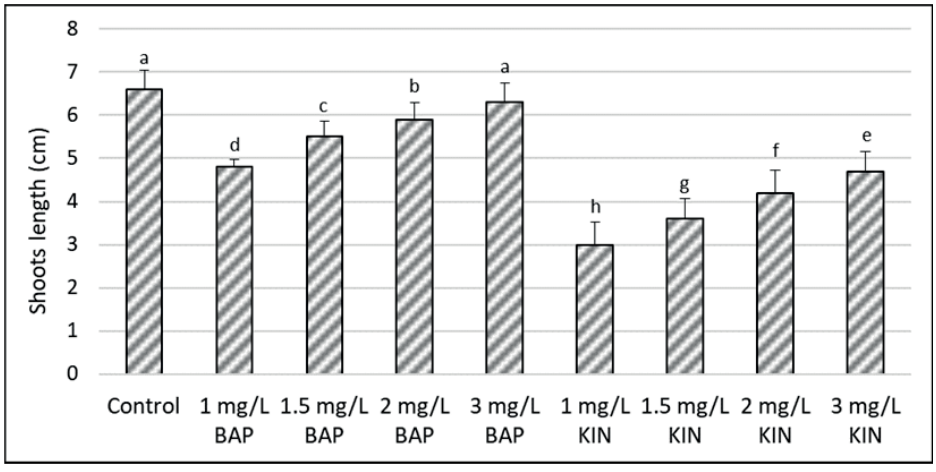

Figure 3. The influence of type and concentration of cytokinin on the shoot lengthof*M. officinalis*.The mean values are accompanied by their corresponding standard deviations; lettersindicate significance of differences as compared to the control at P <0.05

On cytokinin-containing media, the average length of the shoots increased with cytokinin concentration. In all experimental variants, the evaluated indicator recorded significant differences compared to the control at P <0.05 except of the variant in which the culture medium was supplemented with 3 mg/L BAP. In the presence of growth regulators the shoot length was smaller (between 3.0 and 6.3 cm) in all cases compared to the control (6.6 cm)(Figure 3). Sarikhani et al. (2010) achieved good results regenerating lemon balm using MS culturemedia without hormones and induced polyploidy. Ardakani et al. (2003) regenerated *M.officinalis*microplants using a MS culture mediumsupplemented with kinetin (0.2 mg/L), IAA (1 mg/L), 2,4-D (1 mg/L) and coconut juice (15% v/v).

Described results indicate that MS medium supplemented with 3 mg/L BAP was the most effective for regenerationof *M. officinalis* shoots, the regenerated shootsbeinglong and vigorous (Figure 4).

Figure 4. *In vitro*axillary shoot proliferation of *M. officinalis* using a MS medium supplemented with 3 mg/L BAP

After four weeks, the regenerated microshoots were transferred to fresh culture medium that supported the regenerative processes by determining a good proliferation of theshoots.From the qualitative point of view, the biological material resulting from the regeneration of explants had a normal morphology, without vitrification aspects, necrosis or callus differentiation.

Effect of auxin type and concentration on "in vitro" rooting of theshoots

It is known that, in case of *in vitro* cultures, the auxins are responsible for stimulating root development and cell elongation. The effect of auxins on root formation has been well documented in several plant species (Blakesley and Constantine, 1992; Fracaroand Echeverrigary, 2001). Auxins alone or in combination with very low concentration of cytokinins were effective in induction of root primordia (Pierik, 1997).However, high concentrations of auxinsinfluence the development of callus and inhibition of root development.

In the current study, six variants of rooting medium using NAA, IAA and IBA at 0.5 and 1 mg/L were experimented plus a control using a half strength MS basal medium. The type and concentration of auxin on theroot formation of *M. officinalis* was evaluated, in four weeks.

The rooting rate of all treatments resulted to a significant increase compared to the control at P <0.05. The highest value on rooting (96.1%) was obtained at 1 mg/L NAA. Application of IBA or IAA resulted in reduced rooting percentage. For all auxins used, the rooting rate increased with increasing the concentration, but roots were also developed in

an auxin - free medium(40.6% rooted shoots)(Figures5 and 6).This could be possibly to the presence of high amounts of auxins in lemon balm tissues compared to other medicinal plants.

The results were consistent with those obtained by Tavares (1996), reported that root formation required the presence of NAA in the culture medium.Meftahizade et al. (2010b) reported the development of roots at 96%, using 1 mg/L NAA, while by using IBA hormone only 64% of the roots were formed.

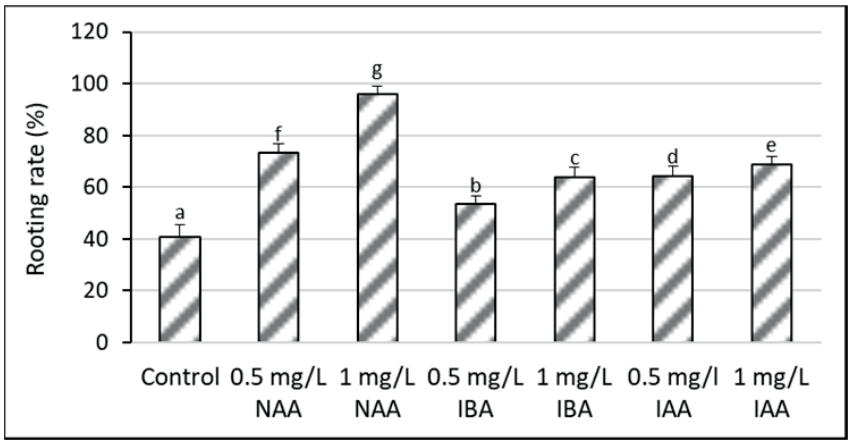

Figure 5. Effect of type and concentration of auxinson the *in vitro* rooting abilityof *M. officinalis* microshoots.The mean values are accompanied by their corresponding standard deviations; lettersindicate significance of differences as compared to the control at P <0.05

Figure 6. *M. officinalis* microshoot rooting on ½MS medium supplemented with 1 mg/L NAA

The microplants regenerated *in vitro*were characterized by long roots with secondary branches, allowing their transplanting to *ex vitro* conditions.

Acclimatization of vitroplants to the"ex vitro" conditions

Acclimatization is the final stage, but most important and necessary for all types of plant micropropagation. Regardless of the *in vitro* culture method adopted, its success depends on the ability to transfer the plants *ex vitro*, to an acceptable economic level. This involves the adaptation of *in vitro* plants to new environmental conditions such as: lower relative humidity, higher light intensity, temperature fluctuations and thestress caused by different diseases.

In vitro regenerated plants had a vigorous root system, supporting a successful passage to the acclimatization phase.

Due to their thin, the leaves plants frequently suffer water losses and this is the reason why their acclimatization is not easy to accomplish, requiring a more humid atmosphere and a controlled temperature, due to major thermic changes must be avoided. The previously mentioned conditions permitted a more facile accommodation period and diminished losses of biological material, the efficiency of the acclimatization plants regenerated *in vitro* was95%.

After the acclimatization and fortification in pots, the plants were transplanted in a greenhouse to continue their growth and development. The plants obtained by *in vitro* propagation have preserved morphological characteristics of the mother stock plants (Figure 7).

Figure 7.*In vitro* regenerated lemon balm plants fortified to pots (a) and soil (b)

We conclude that the results obtained throughout *in vitro* regeneration phases certify that for *M. officinalis*, the micropropagation technique represents an advantageous alternative to the classic methods of propagation, which allows the rapid and massive propagation of high quality clonal plants.

CONCLUSIONS

The research shows that *in vitro* shoot multiplication of *M. officinalis* is depending upon the treatment with growth regulators that are used. The newclonal plants obtained by micropropagation have been appeared normal and no morphological variation was shown. The *in vitro* culture system was successfully established for lemon balmand offers a viable tool for preservation, multiplication and sustainable production of this very valuable medicinal species. This protocol can ensure a stable supply of this commercial crop in a limited time and space, irrespective of seasonal variations and thus meet the global demand for its essential oil. The regenerated plants could also serve as potential sources for the extraction of active compounds for pharmaceutical purposes.

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VEGETABLE GROWING

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ASPECTS REGARDING MONITORING OF THE PEST *TUTA ABSOLUTA* (TOMATO LEAF MINER) ON TOMATO CROPS UNDER HIGH PLASTIC TUNNELS AND EFFICACY OF SOME INSECTICIDES TESTED FOR ITS CONTROL

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ABSTRACT

***Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a pest native from South America, which was first reported in Romania in 2009 (Satu Mare). Among the vegetable species, tomatoes are the main host of the pest, but can also be found on potato, black nightshade (*Solanumnigrum*), eggplant, bell peppers and tobacco.Yield losses caused by *Tuta absoluta* can reach 100%, in the absence of adequate control measures.Pheromone traps are a complementary method of this pest control, contributing considerably to reducing its population. The experiments were done in 2020, in the area of influence of R.I.V.F.G. - Vidra, in the first cycle of crop, using the hybrids Sahmat F1 and Zadurella F1.The Delta traps with pheromone were placed in 2 high plastic tunnels with tomatoes, from 2 different locations (Dobreni and Vărăşti) and the number of captured adults and the frequency and intensity of the attack on foliage and fruits were periodically recorded, between May and July.The number of captured adults on the sticky plate which have 397,75 cm^2, ranged in time, between 52 and 482. Chemical treatments were also applied, at 7 daysintervals, to prevent the spread of the pest and limit the damage caused by it. The average yields obtained were between 6.950 kg/m^2 (farmer 1, Dobreni) and 8.270 kg/m^2 (farmer 2, Vărăşti).**

Keywors: attack, Delta trap, pheromone, treatments, yield

INTRODUCTION

Tuta absoluta (tomatoleafminer) is a pest native from South America that later spread to most tomato-growing countries.The pest has about 12 generations of adults per year (Mkonyi et al., 2020).

In Romania, was first reported in 2009, in the north part of the country (Satu Mare), and a year later the presence of this pest was reported in tomato crops in glasshousesaround Bucharest (Bratu et al., 2015).

The main method of control is the use of chemicals, but alternative, less polluting control methods have also been promoted, such as: microbiological control and biological control by releasing parasites and predators.Other complementary methods of control are the use of light traps and traps with asyntheticpheromones to annihilate males by disturbing mating.Using traps with synthetic pheromones, the ability of males to identify females is disturbed and implicitly the density of the pest population is considerably reduced (Cocco et al., 2012).

MATERIALS AND METHODS

The experiments were done in 2020, in the first cycle of crop, at 2 farmers from Dobreni and Vărăștivillages, Giurgiu county, located in the area of influence of R.I.V.F.G. - Vidra, by placing Delta traps, with pheromones, in tomato crops from high plastic tunnels. In the first location, the hybrid used was Sahmat F1, a rustic hybrid of tomatoes, with undetermined growth, recommended for spring crops in glasshouses, high plastic tunnels, but also in the field, to obtain early harvests (www.marcoser.ro), and in the second location was planted the hybridZadurella F1, an early hybrid, with undetermined growth, recommended for protected areas (www.agrobro.ro). The planting took place on 09.05.2020 and 12.03.2020, respectively.Were kept 7 fruit sets (farmer 1) and 8 fruit sets (farmer 2).
The traps were placed in tomato crops on 20.05.2020. In the case of the first farmer (Dobreni), on the same day, the first chemical control treatment was applied. During the experiment, 8 treatments were applied, at 7 daysintervals, with several control products, alternatively, as follows: Coragen 0.0175%, Alverde 0.1%, Affirm 0.15%, Benevia 0.0125%, Laser 240 SC 0.05%, Coragen 0.0175%, Benevia 0.0125% and VoliamTargo 0.08%. 9 treatments were applied to the second farmer (Vărăști), at 7 daysintervals, as follows: Alverde 0.1%, Coragen 0.0175%, Affirm 0.15%, VoliamTargo 0.08 %, Laser 240 SC 0.05%, Coragen 0.0175%, Alverde 0.1%, Laser 240 SC 0.05% and Affirm 0.15%.
Pest monitoring was done weekly, by changing the sticky plates with pheromones from Delta traps, on the dates: 10.06, 17.06, 24.06, 01.07, 08.07, 15.07 at the first farmer and, respectively, 03.06, 10.06, 17.06, 24.06, 01.07, 08.07, 15.07 to the second farmer. Dynamic observations were made on the frequency and intensity of the pest attack, finally calculating, according to the Abbot`s formula, the degree of attack on the leaves. Yield was also recorded, reported at the end/m^2.

RESULTS AND DISCUSSIONS

On May 20, when the Delta traps were placed in the tomato crops, the pest was present on both high plastic tunnels of the 2 farmers, the attack being only on the leaves (fig. 1).

Figure 1. *Tuta absoluta* attack on tomato leaf

Figure 2. *Tuta absoluta* attack on tomato fruit

At farmer 1, the attack on fruits (fig. 2) was registered on 01.07 (table 1), while on farmer 2 on 17.06 (table 2).

During the crop cycle, the sticky plates were replaced when their surface was full of captured adults.At the first farmer, their number varied between 52 and 482(fig. 3), with an average of 374 captured adults/397.75 cm^2 (94.0 adults/100 cm^2).The degree of attack evolved from 0.3% (27.05) to 16.1% (22.07; table 1).

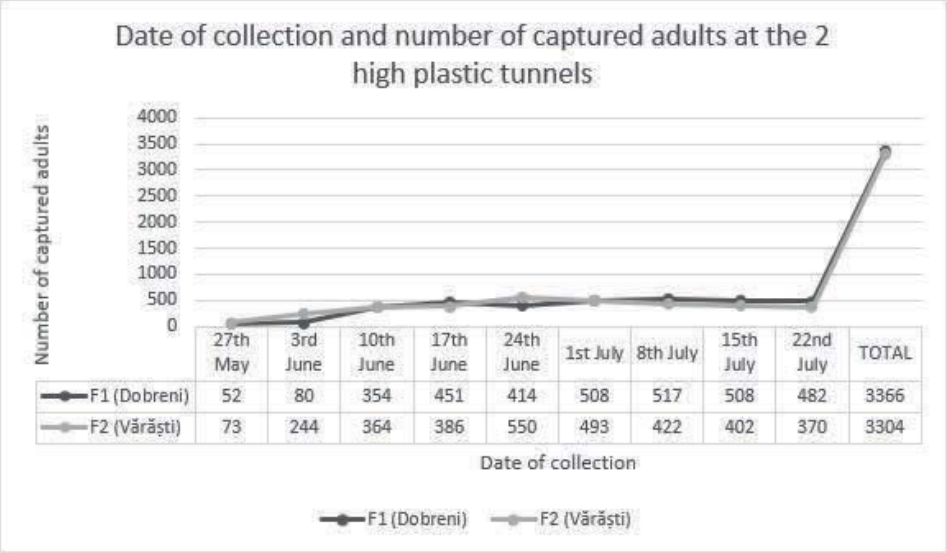

Figure 3. The date of collection of pheromone plates and the number of adults caught between May and July at the 2 high plastic tunnels

Table 1. Evolution of the attack of *Tutaabsoluta*peston tomato crop from the high plastic tunnel (farmer 1, Dobreni)

Date of monitoring	Number of captured adults		Degree of attack (%)	Frequency of attacked fruits (%)
	on plate (397,75 cm^2)	on 100 cm^2		
27.05	52	13.1	0.3	0
03.06	80	20.1	0.7	0
10.06	354	89.0	1.5	0
17.06	451	113.4	1.7	0
24.06	414	104.1	3.9	0
01.07	508	127.7	5.4	1.1
08.07	517	130.0	8.6	2.9
15.07	508	127.7	11.8	5.9
22.07	482	121.2	16.1	8.1
Average	374	94.0	5.5	4.5

At the second farmer, the number of adults varied between 73 and 370 (fig. 3), with an average of 367 captured adults/397.75 cm^2 (92.3 adults/100 cm^2). The degree of attack evolved from 0.2% (27.05) to 11.9% (22.07; table 2).

Figure 4. Sticky plates with captured adults and the crops aspect on 3 different dates (27.05; 24.06; 22.07), at farmer 1

Based on the results obtained, at farmer 2 finds that the pest density was lower, and consequently the degree of attack was lower (11.9%) than the first farmer (16.1%). The average number of fruits attacked on the second farmer was lower (3.0%) compared to the first farmer (4.5%).

Towards at the end of the growing period, there was a decline in the number of adults caught on the sticky plates (tables 1 and 2), probably due to the migration of the pests to young plants, newly established in the crops from the second cycle.

Table 2. Evolution of the attack of *Tutaabsoluta* pest on the tomato crop from the high plastic tunnel (farmer 2, Vărăşti)

Date of monitoring	Number of captured adults		Degree of attack (%)	Frequency of attacked fruits (%)
	on plate (397,75 cm^2)	on 100 cm^2		
27.05	73	18.3	0.2	0
03.06	244	61.3	1.3	0
10.06	364	91.5	2.5	0
17.06	386	97.0	3.2	0.8
24.06	550	138.3	4.5	1.3
01.07	493	123.9	5.8	1.8
08.07	422	106.1	7.7	3.2
15.07	402	101.1	9.1	4.7
22.07	370	93.0	11.9	6.2
Average	367	92.3	5.1	3.0

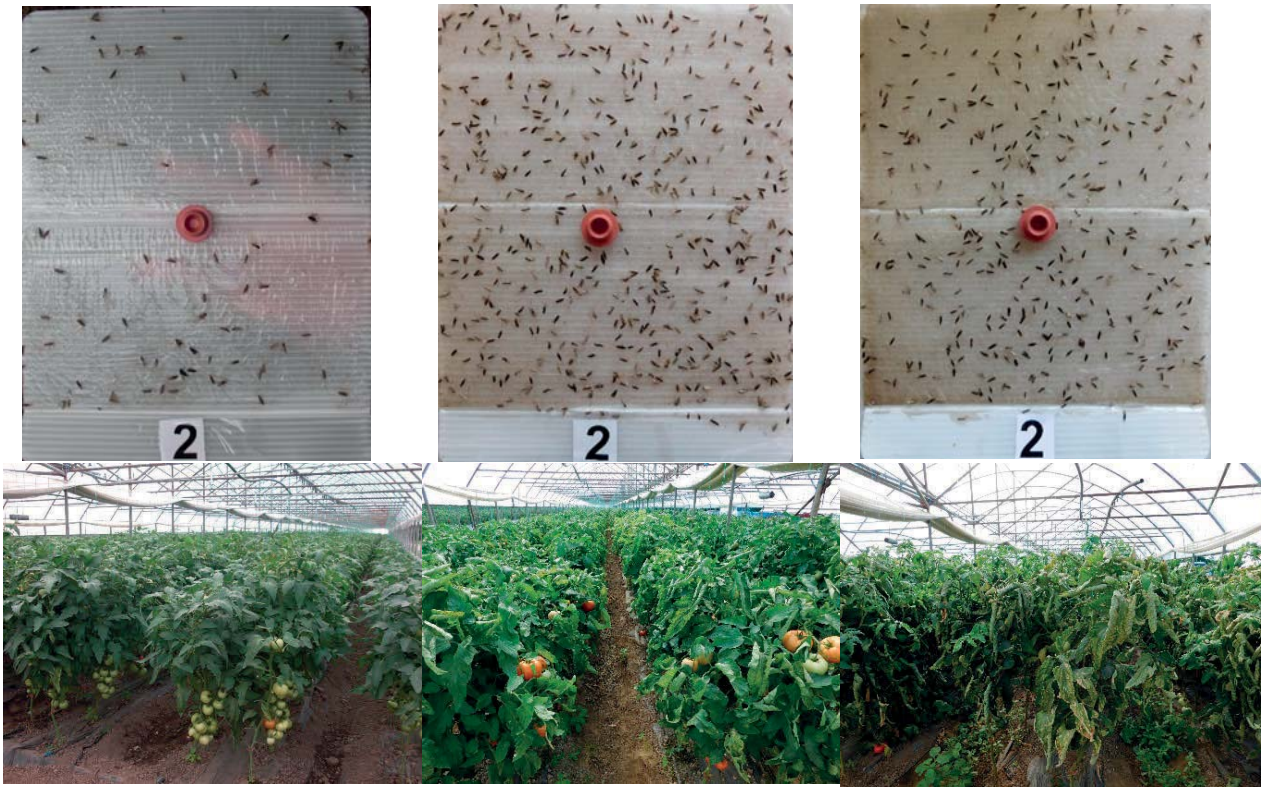

Figure 5. Sticky plates with captured adults and the crops aspect at 3 different dates (27.05; 24.06; 22.07) at farmer 2

In figures 4 and 5 is shown the aspect of tomato crops, from the 2 farmers, in different phenological phases during May-July and the sticky collector plates.

The yields obtained at the end of the growth period were 6,950 kg/m^2 (69.5 t/ha) for farmer 1 and 8,270 kg/m^2 (82.7 t/ha) for farmer 2, respectively.

CONCLUSIONS

- Delta pheromone traps are especially useful for signaliging the appearance of the pest *Tuta absoluta* and monitoring the evolution of its attack on protected tomato crops, being an important decision-maker in the establishing the moment when must to start the treatments.
- At the same time, they contribute significantly to the decrease of pest populations in a certain habitat, by replacing them at the right time (depending on population density) and the use number of recomendedtrapsper unit area (3-4 traps/ha).
- Based on the results obtained in the experiments performed on 2 farmers, from Dobreni and Vărăşti respectively, it can be concluded that, by using Delta pheromone traps and applying treatments with specific products to control this pest, at intervals dictated by the pest density (of 7 days), the pest populations can be kept under control, which provide to obtain constant and good quality tomato yields.

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POSSIBILITIES OF COMPLEX CONTROL OF PATHOGENS AND PEST ON TOMATO CROPS UNDER HIGH PLASTIC TUNNELS

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ABSTRACT

The experiment was carried out in 2020, under high plastic tunnels conditions, using the tomato hybrid Prekos F1 and aimed to evaluate the efficacy of different combinations of fungicides with insecticides for complex control of pathogens *Alternaria solani* (early blight), *Fulvia fulva* (leaf mold) and pests *Tuta absoluta* (tomato leaf miner) and *Helicoverpa armigera* (cotton bollworm). The experimental variants were: 1. Cabrio Top 0.2% + Coragen 0.0175%; 2. Cabrio Top 0.2% + Voliam Targo 0.08%; 3. Dagonis 0.1% + Coragen 0.0175%; 4. Dagonis 0.1% + Voliam Targo 0.08%; 5. Cidely Top 0.1% + Coragen 0.0175%; 6. Cidely Top 0.1% + Voliam Targo 0.08%; 7. Ortiva Top 0.1% + Coragen 0.0175%; 8. Ortiva Top 0.1% + Voliam Targo 0.08%; 9. Untreated control. Six foliar treatments were applied at 10-days intervals. The efficacy of these combinations of fungicides with insecticides was assessed according to the degree of attack (%) for *Alternaria solani*, *Fulvia fulva* and *Tuta absoluta* or according to the frequency of the attacked fruits (%) for *Helicoverpa armigera* and was between 85.0% and 90.7%. The highest yields were registered in variants 1 (Cabrio Top 0.2% + Coragen 0.0175% - 5,535 kg/m^2), 3 (Dagonis 0.1% + Coragen 0.0175% - 5,440 kg/m^2) and 2 (Cabrio Top 0.2% + Voliam Targo 0.08% - 5,362 kg/m^2) as compared to 4,490 kg/m^2 in the untreated control variant. The differences of yield, obtained in addition to the untreated control variant, were very significant in all cases.

Keywords: fungicides, insecticides, combinations, efficacy, yields

INTRODUCTION

Among the vegetable species cultivated in Romania, tomatoes occupy the first place, with 40,734 ha (FAO, 2018). From this area ≈8000 ha are occupied with crops under areas (high plastic tunnels and glasshouses). Also, in this case, tomatoes are the predominant species, their cultivation being practiced on ≈70% of the surface in cycle I, cycle II or extended cycle.
The climatic conditions under protected areas, necessary for the growth and development of tomato plants are, at the same time, favorable for the appearance and evolution of the attack of harmful agents of economic importance, among which we mention: *Alternaria solani* (early blight), *Botrytis cinerea* (gray mold), *Fulvia fulva* (leaf mold), *Erysiphe* sp. (powdery mildew), *Colletotrichum coccodes* (anthracnose), *Phytophthora infestans* (late blight), *Tuta absoluta* (tomato leaf miner), *Helicoverpa armigera* (cotton bollworm), *Macrosiphum euphorbiae* (tomato aphid), *Thrips tabaci* (common thrips), etc. Studies undertaken worldwide and in Romania have led to the establishment of elements of epidemiology and

bioecology of harmful organisms and, at the same time, to the development of the most appropriate control measures. In connection with foliar pathogens that attack tomato crops, strategies have been established to control them with fungicides (Miller et al., 2002; Costache et al., 2018). Regarding the *Tuta absoluta* and *Helicoverpa armigera* pests, there are numerous studies on bioecology and economic importance and the measures and means of its integrated control have been established (Desneux et al., 2010; Bratu et al., 2015; Hogea, 2020; Sene et al., 2020). The experiments carried out at ICDLF - Vidra aimed to identify combinations of fungicides with insecticides designed to control the complex of pathogens and pests present at the same time in tomato crops under high plastic tunnels.

MATERIALS AND METHODS

The experiment was carried out in 2020, under high plastic tunnels, protected with "insect proof" net, in the first cycle of crop, using the tomato hybrid Prekos F1 and included 9 experimental variants, placed according to the method of randomized blocks.
The experimental variants were:
1. Cabrio Top 0.2% + Coragen 0.0175%
2. Cabrio Top 0.2% + Voliam Targo 0.08%
3. Dagonis 0.1% + Coragen 0.0175%
4. Dagonis 0.1% + Voliam Targo 0.08%
5. Cidely Top 0.1% + Coragen 0.0175%
6. Cidely Top 0.1% + Voliam Targo 0.08%
7. Ortiva Top 0.1% + Coragen 0.0175%
8. Ortiva Top 0.1% + Voliam Targo 0.08%
9. Untreated control.

During the vegetation period, 6 foliar treatments were applied at 10-days intervals, as follows: June 12 (T1), June 22 (T2), July 2 (T3), July 13 (T4), July 23 (T5)) and August 3 (T6). Table 1 summarizes the plant protection products used in this experiment.

Table 1. Phytosanitary products used in the experiment

Phytosanitary products	Active ingredients	Mode of action	Pathogens and pest controled	Break time (days)
Cabrio Top	piraclostrobin 5% + metiram 55%	contact and translaminar	*Alternaria solani, Fulvia fulva, Phytophthora infestans, Leveillula taurica, Septoria lycopersici*	7
Dagonis	difenoconazol 50 g/l + fluxapiroxad 75 g/l	contact and systemic	*Alternaria solani, Fulvia fulva, Leveillula taurica*	3
Cidely Top	difenoconazol 125 g/l + ciflufenamid 15 g/l	contact and systemic	*Alternaria solani, Fulvia fulva, Leveillula taurica*	3
Ortiva Top	azoxistrobin 200 g/l + difenoconazol 125 g/l	contact and systemic	*Alternaria solani, Botrytis cinerea, Fulvia fulva, Phytophthora infestans, Leveillula taurica*	7
Coragen	clorantraniliprol 20%	local systemic and translaminar	*Tuta absoluta, Helicoverpa armigera*	1
Voliam Targo	abamectin 18g/l + clorantraniliprol 45 g/l	local systemic and translaminar	*Tuta absoluta, Helicoverpa armigera, Liriomyza* sp., *Tetranychus urticae*	3

During the vegetation period, dynamic observations were made on the appearance and evolution of the attack of pathogens and pests (frequency and intensity of attack) in correlation with climatic factors, and finally the degree of attack was calculated (%; for *Alternaria solani, Fulvia fulva, Tuta absoluta*) or frequency of fruits attack (%; for *Helicoverpa armigera*) and efficacy (%) of combinations of plant protection products used to control harmful organisms.
The yield was also harvested in dynamics, on variants and repetitions, and the obtained data were processed by the method of analysis of variance.

RESULTS AND DISCUSSIONS

The attack of the pathogens *Alternaria solani, Fulvia fulva* and the pests *Tuta absoluta* (fig. 1 and 2) and *Helicoverpa armigera* appeared and evolved in the tomato crop.

The attack produced by *Alternaria solani* appeared on May 22 and evolved slowly so that, in the first decade of August, the degree of attack reached 8.2% (Table 2). The *Fulvia fulva* attack started on May 28, the value of the degree of attack reaching 31.2% at the end of the first decade of August. The attack was favored by high average temperatures (27.9-29.9 ° C) and maximum relative humidity over 90%.

Table 2. The appearance and evolution of the attack of pathogens and pests in correlation with climatic factors

Pathogens/Pests/ climatic factors	Date of attack appearance	Degree of attack (%) / attack frequency (%)								
		May		June			July			August
		Decade		Decade			Decade			Decade
		II	III	I	II	III	I	II	III	I
Alternaria solani	22.05	0	0.2	0.6	1.7	2.9	3.6	4.2	6.7	8.2
Fulvia fulva	28.05	0	0.4	0.8	1.7	3.9	9.6	15.8	23.3	31.2
Tuta absoluta	14.05	0.9	1.5	3.8	6.1	10.2	17.3	28.7	35.4	40.6
Helicoverpa armigera	22.06	0	0	0	0	0.6	1.2	2.9	4.7	7.8
Minimum temperature (°C)	-	14.5	10.4	12.2	16.8	16.3	17.9	15.5	18.1	17.6
Average temperature (°C)	-	26.1	20.2	23.5	24.9	27.9	29.7	27.0	29.6	29.9
Maximum temperature (°C)	-	39.4	34.5	37.2	37.8	40.6	41.7	39.6	43.0	42.2
Minimum humidity (%)	-	25.2	31.8	30.4	42.1	30.2	25.8	24.8	24.6	18.6
Average humidity (%)	-	54.6	66.1	64.3	74.8	62.7	55.9	54.8	58.7	44.4
Maximum humidity (%)	-	86.2	93.1	93.7	96.7	93.9	89.9	88.3	91.9	77.9

Among the pests was the earliest attack of *Tuta absoluta* (May 14) which evolved rapidly due to high average temperatures (27.9-29.9 ° C), from the third decade of June - the first decade of August, reaching at 40.6%. The latest attack was the pest *Helicoverpa armigera* (June 22), the frequency of the attack reaching 7.8%, at the end of the first decade of August.

The average efficacy of the combinations of fungicides with insecticides experimented for the complex control of pathogens and pests was between 85.2% (variant 8: Ortiva Top 0.1% + Voliam Targo 0.08%) and 90.7% (variant 1: Cabrio Top 0.2% + Coragen 0.0175%; table 3). Based on the results obtained, it can be appreciated that practically all the combinations tried ensured a good protection of the tomato plants against

the attack of the pathogens *Alternaria solani, Fulvia fulva* and the pests *Tuta absoluta* and *Helicoverpa armigera*.

Regarding the obtained yield, it varied between 5.105 kg/m^2 (V6) and 5.535 kg/m^2 (V1) as compared to 4.490 kg/m^2 in the untreated control variant (Table 4). The highest yields were registered at variants 1 (5.535 kg/m^2), 3 (5.440 kg/m^2) and 2 (5.362 kg/m^2). The yield differences obtained in addition to the untreated control variant, varied between 0.615 kg/m^2 at V6 and 1.045 kg/m^2 at V1 are very significant.

Table 3. Efficacy of product combinations in the complex control of pathogens and pests on tomato crop under high plastic tunnels

Variant	Degree of attack (%)			Frequency of attack (%)	Eficacy (%)				Average efficacy (%)
	A. solani	***F. fulva***	***T. absoluta***	***H. armigera***	***A. solani***	***F.fulva***	***T. absoluta***	***H. armigera***	
1. Cabrio Top 0.2% + Coragen 0.0175%	0	3.5	3.2	1.4	100.0	88.8	92.1	82.0	90.7
2. Cabrio Top 0.2% + Voliam Targo 0.08%	1.2	3.8	5.2	1.6	85.4	87.8	87.2	79.5	85.0
3. Dagonis 0.1% + Coragen 0.0175%	0	3.6	3.8	1.6	100.0	88.5	90.6	79.5	89.6
4. Dagonis 0.1% + Voliam Targo0.08%	0	4.0	4.6	2.2	100.0	87.2	88.7	71.8	86.9
5. Cidely Top 0.1% + Coragen 0.0175%	1.4	3.8	3.1	1.4	82.9	87.8	92.4	82.0	86.3
6. Cidely Top 0.1% + Voliam Targo 0.08%	0	5.1	4.5	1.3	100.0	83.6	88.9	83.3	88.9
7. Ortiva Top 0.1% + Coragen 0.0175%	1.6	6.1	3.9	0	80.5	80.4	90.4	100,0	87.8
8. Ortiva Top 0.1% + Voliam Targo 0.08%	1.2	5.4	3.3	1.5	85.4	82.7	91.9	80.8	85.2
9. Martor netratat	8.2	31.2	40.6	7.8	-	-	-	-	-

Table 4. The influence of treatments with combinations of fungicides and insecticides on tomato yield under high plastic tunnels (Prekos F1 hybrid)

Variant	Yield (kg/m²)	Relative yield (%)	Difference from control (kg/m²)	Significance
1	5.535	123.2	+1.045	***
2	5.362	119.4	+0.872	***
3	5.440	121.1	+0.950	***
4	5.308	118.2	+0.818	***
5	5.155	114.8	+0.665	***
6	5.105	113.6	+0.615	***
7	5.355	119.2	+0.865	***
8	5.260	117.1	+0.770	***
9	4.490	100.0	-	-

DL 5%=0.30; DL 1%=0.42; DL 0,1%=0.56

CONCLUSIONS

In the first cycle, the attack of the pathogens *Alternaria solani, Fulvia fulva* and the pests *Tuta absoluta* and *Helicoverpa armigera* was manifested in the tomato crop under high plastic tunnels.

The efficacy of the combinations of fungicides with experienced insecticides, used to complex control of harmful organisms varied between 85.2% (V 8: Ortiva Top 0.1% + Voliam Targo 0.08%) and 90.7% (V1: Cabrio Top 0.2% + Coragen 0.0175%).

The highest yields were registered for variants 1 (Cabrio Top 0.2% + Coragen 0.0175%; 5.535 kg/m²), 3 (Dagonis 0.1% + Coragen 0.0175%; 5.440 kg/m²) and 2 (Cabrio Top 0.2% + Voliam Targo 0.08%; 5.362 kg/m²).

The yield differences obtained in addition to the untreated control variant were, in all cases, very significant.

Figure 1. *Tuta absoluta*attack on the untreated control

Figure 2. *Fulvia fulva* and *Tuta absoluta* attack on the untreated control

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ACTIVE INGREDIENTS COMBINATIONS FOR PATHOGENS AND PESTS CONTROL ON EGGPLANT CROPS IN THE FIELD

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ABSTRACT

The present study aims to identify combinations of active substances for the complex control of pathogens and pests on eggplant crops in the field. The experiments were conducted between 2016 - 2017, in field conditions, using the variety of eggplant Luiza and the following experimental variants: V 1 - Acrobat MZ 69 WG 0.2% + Mospilan 20 SG 0.04%; V 2 - Acrobat MZ 69 WG 0.2% + Vertimec 1.8 EC 0.1%; V 3 - Acrobat MZ 69 WG 0.2% + Laser 240 SC 0.05%; V 4 - Melody Compact 49 WG 0.2% + Mospilan 20 SG 0.04%; V 5 - Melody Compact 49 WG 0.2% + Vertimec 1.8 EC 0.1%; V 6 - Melody Compact 49 WG 0.2% + Laser 240 SC 0.05%; V 7 - Ortiva Top 0.1% + Mospilan 20 SG 0.04%; V 8 - Ortiva Top 0.1% + Vertimec 1.8 EC 0.1%; V 9 - Ortiva Top 0.1% + Laser 240 SC 0.05%; V 10 - untreated control. The average efficacy of the treatment variants experienced in 2016 and 2017 varied between 83.2% (V4) and 85.0% (V8) depending on the combinations of products pathogens and pests control. Analyzing the yields obtained (34.6 -44.0 t / ha in 2016 and 33.5 - 43.1 t / ha in 2017), compared to the untreated control variant (30.4 t / ha in 2016 and 31.1 t / ha in 2017), it is found that the yield differences obtained in addition to the untreated control variants were in all cases, very significant.

Keywords: early blight, gray mold, fruit rot, Colorado potato beetle, the common red spider, green nightshade lice, fruit caterpillar

INTRODUCTION

Eggplants (*Solanum melongena* L.) are among the most cultivated vegetable species worldwide, including in Romania. According to statistical data provided by FAO (2020), the area cultivated with eggplant in Romania was in 2018 of 9025 ha, and the total yield of 152719 tons. Eggplants are attacked by numerous pathogens and pests (Costache M., Roman T., 2007) of which we mention *Alternaria porri* f.sp.*solani* (early blight), *Botrytiscinerea* (gray mold), *Phytophthora parasitica* (fruit rot; Buzatu et. al., 2017) and *Leptinotarsa decemlineata* (Colorado beetle), *Macrosiphum euphorbiae* (green nightshade lice), *Tetranych*us *urticae* (the common red spider) and *Helicoverpa armigera* (fruit caterpillar). *Alternaria porri* f. sp. *solani* occur frequently in eggplant crops, especially in

years with high average temperatures and heavy rainfall. Disease symptoms are characteristic dark brown to black lesions with concentric rings, which produce a 'target spot' effect (Cristea, 2005). *Botrytis cinerea* occurs frequently in crops of eggplants in protected areas, but also in the fields in years with abundant precipitation or if the culture is irrigated by sprinkling irrigation. On fruits, at different stages of development, the attack usually begins at the site of insertion of the peduncle and from here progresses to their tip. The attacked tissues discolor and turn purple, with a gray tinge. *Phytophthora parasitica* usually attacks mature fruits in the basal part that touch the ground or are close to it. On their surface appear large, brown spots, concentrically zoned, bordered by an obvious area of lighter color. Although the whole plant may be susceptible, fruit rot is the main symptom caused by *Phytophthora parasitica* in eggplants (Roberts & al., 2008). *Leptinotarsa decemlineata* (Colorado potato beetle) attacks in the larval and adult stages, affecting all the aerial organs of the plants, with preferential location on the foliage. The common red spider (*Tetranychus urticae*) affects all the aerial organs of the plants and preferably the foliar leaves, being located on the lower part. Hossain et al. (2006) and Naher (2005) observed that by applying acaricides, the population of *T. urticae* in the field was drastically reduced. Sayed et al. (2006) found that Vertimec is more effective than Actellic and Biofly against *T. urticae*. *Macrosiphum euphorbiae* (green nightshade lice) is common in dry and hot years, forming colonies clustered on the underside of leaves and scattered on flowers. The young larvae of *Helicoverpa armigera* (fruit caterpillar) develop on leaves and flowers, after which they enter into the fruit where they consume the seeds and leave numerous droppings. The attacked fruits become unfit for consumption.
The experiments organized at R.I.V.F.G Vidra aimed to prevent the occurrence and control of the attack of pathogens and pests in the crop of eggplant by using combinations of active substances.

MATERIALS AND METHODS

The experiments were carried out in 2016 and 2017, in field conditions, using the Luiza eggplant variety. The products used to control pathogens and pests in eggplant field crops are shown in table 1.

Table 1. Products used for pathogens and pests control of eggplant crop in the field (Vidra, 2016 - 2017)

Product and concentration	Active ingredients	Mode of action	Break time (days)
Fungicides			
Acrobat MZ 69WG 0,2 %	dimethomorph 9% + mancozeb 60%	translaminar, local systemic + contact	7
Melody Compact 49 WG 0,2 %	iprovalicarb 8.4% + Cu as oxychloride Cu 40.6%	systemic, contact	7
Ortiva Top 0,1 %	azoxystrobin 200 g / l + diphenoconazole 125 g / l	contact, local systemic, translaminar	7
Insecticides - acaricides			
Mospilan 20 SG 0,04 %	acetamiprid 20 %	systemic	3
Vertimec 1,8 EC 0,1 %	abamectin 18 g/l	systemic, translaminar, penetrating, ingestion	3
Laser 240 SC 0,05 %	spinosad 240 g/l	contact, ingestion	3

The organized experience was of a single-factor type, placed in randomized blocks, with 10 variants, in 3 replications. The crop was established on May 16, 2016 and on May 12, 2017, on land mulched with black polyethylene foil. The size of the repetition plot was 7.5 m^2, having a number of 24 plants, arranged in 2 rows, 70 cm apart and 40 cm between plants on the row. The experimental variants were the following: V 1 - Acrobat MZ 69 WG 0.2% + Mospilan 20 SG 0.04%; V 2 - Acrobat MZ 69 WG 0.2% + Vertimec 1.8 EC 0.1%; V 3 - Acrobat MZ 69 WG 0.2% + Laser 240 SC 0.05%; V 4 - Melody Compact 49 WG 0.2% + Mospilan 20 SG 0.04%; V 5 - Melody Compact 49 WG 0.2% + Vertimec 1.8 EC 0.1%; V 6 - Melody Compact 49 WG 0.2% + Laser 240 SC 0.05%; V 7 - Ortiva Top 0.1% + Mospilan 20 SG 0.04%; V 8 - Ortiva Top 0.1% + Vertimec 1.8 EC 0.1%; V 9 - Ortiva Top 0.1% + Laser 240 SC 0.05%; V 10 - untreated control.
The physical compatibility of the product mixtures was determined in the laboratory. Three treatments were performed during the growing season. The first treatment was done at the beginning of the risk of infection and infestation and the others at intervals of 7-10 days. During the vegetation period, observations were made regarding the occurrence and evolution of the attack of pathogens: frequency (%) and severity (%) of the attack and the degree of attack (%) and efficacy (%) were calculated. The degree of attack was calculated with the formula (F % x S %) / 100 and the efficacy with the formula (untreated DA% - treated DA%) x 100 / untreated DA. Observations were also made on the yield, by weighing the fruits on variants and repetitions at harvest.
Yield data were statistically processed by analysis of variation (ANOVA), and the level of significance of yield differences was interpreted with the Fisher test: °°° = Non significant; ° =significant; * = distinct significant; *** = very significant.

RESULTS AND DISCUSSIONS

In the experimental crops of eggplant in the field, the attack of the following pathogens and pests was manifested in 2016 and 2017: *Alternaria porri* f.sp. *solani* (early blight), *Botrytis cinerea* (gray mold), *Phytophthora parasitica* (fruit rot), *Leptinotarsa decemlineata* (Colorado beetle), *Macrosiphum euphorbiae* (green nightshade lice), *Tetranychus urticae* (common red spider).

The combinations of products tested were noted for their good efficacy in controlling the following harmful organisms (Tables 2 and 3):

1. Acrobat MZ 69 WG + Mospilan 20 SG: for *Alternaria porri* f.sp. *solani,Botrytis cinerea, Phytophthora parasitica, Leptinotarsa decemlineata* and *Macrosiphum euphorbiae* (average E = 83.6% - 2016; 83.4% - 2017; 83.5% - average value 2016-2017);
2. Acrobat MZ 69 WG + Vertimec 1.8 EC: for *Alternaria porri* f.sp. *solani,Botrytis cinerea, Phytophthora parasitica* and *Tetranychus urticae* (average E = 85.3% - 2016; 84.4% - 2017; 84.8% - average value 2016-2017);
3. Acrobat MZ 69 WG + Laser 240 SC: for *Alternaria porri* f.sp. *solani,Botrytis cinerea, Phytophthora parasitica* and *Helicoverpa armigera* (average E = 84.2% - 2016; 84.2% - 2017; 84.2% - average value 2016-2017);
4. Melody Compact 49 WG + Mospilan 20 SG: for *Alternaria porri* f.sp*. solani*, *Botrytis cinerea, Phytophthora parasitica, Leptinotarsa decemlineata* and *Macrosiphum euphorbiae* (average E = 83.1% - 2016; 83.4% - 2017; 83.2% - average value 2016-2017);
5. Melody Compact 49 WG + Vertimec 1.8 EC: for *Alternaria porri* f.sp. *solani*, *Botrytis cinerea, Phytophthora parasitica* and *Tetranychus urticae* (average E = 84.2% - 2016; 84.37% - 2017; 84.2% - average value years 2016-2017);

6. Melody Compact 49 WG + Laser 240 SC: for *Alternaria porri* f.sp. *solani, Botrytis cinerea, Phytophthora parasitica* and *Helicoverpa armigera* (average E = 83.7% - 2016; 83.4% - 2017; 83.5% - average value 2016-2017);

7. Ortiva Top + Mospilan 20 SG: for *Alternaria porri* f.sp. *solani, Botrytis cinerea, Phytophthora parasitica, Leptinotarsa decemlineata* and *Macrosiphum euphorbiae* (average E = 83.7% - 2016; 85.0% - 2017; 84.35% - average value 2016-2017);

8. Ortiva Top + Vertimec 1.8 EC: for *Alternaria porri* f.sp. *solani*, *Botrytis cinerea, Phytophthora parasitica* and *Tetranychus urticae* (average E = 85.5% - 2016; 85.0% - 2017; 85.0% - average value 2016-2017);

9. Ortiva Top + Laser 240 SC: for *Alternaria porri* f.sp. *solani, Botrytis cinerea, Phytophthora parasitica* and *Helicoverpa armigera* (average E = 84.1% - 2016; 83.2% - 2017; 83.6% - average value 2016-2017);

The average efficacy of the treatment variants experienced in 2016 and 2017 varied between 83.2% (V4) and 85.0% (V8) depending on the combination of products pathogens and pest control.

Table 2. The efficacy of some combinations of fungicides with insecticides - acaricides in controling of pathogens and pests in the eggplant crop in the field (Vidra, 2016)

Variant	Pathogens and pests														Average efficacy (%)*
	Alternaria porri f.sp *solani*		*Botryris cinerea*		*Phytophthora parasitica*		*Leptinotarsa decemlineata*		*Macrosiphum euphorbiae*		*Tetranychus urticae*		*Helicoverpa armigera*		
	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)	
V1.	2.0	85.8	2.3	79.5	1.5	88.2	2.0	79.2	1.3	85.4	4.1	49.4	3.6	63.3	83.6
V2.	2.1	85.1	2.4	78.6	1.6	87.4	4.5	53.1	3.5	60.7	0.8	90.1	4.1	58.2	85.3
V3.	2.3	83.7	2.6	76.8	1.3	89.8	4.7	54.0	3.0	66.3	4.5	44.4	1.3	86.7	84.2
V4.	2.5	82.3	2.4	78.6	1.1	91.3	1.7	82.3	1.7	80.9	4.4	45.7	3.9	60.2	83.1
V5.	2.7	80.8	2.6	76.8	1.4	89.0	4.8	50.0	3.8	57.3	0.8	90.1	4.2	57.1	84.2
V6.	2.4	83.0	2.7	75.9	1.5	88.2	5.1	46.9	3.4	61.8	4.5	44.4	1.2	87.7	83.7
V7.	2.2	84.4	1.5	86.6	2.1	83.5	1.9	80.2	1.5	83.1	3.9	51.8	3.7	62.2	83.7
V8.	2.5	82.3	1.6	85.7	1.9	85.0	4.7	51.0	3.9	56.2	0.9	88.9	4.4	55.1	85.5
V9.	2.3	83.7	1.8	83.9	2.4	81.1	5.3	44.8	3.3	62.9	4.6	43.2	1.2	87.7	84.1
V10.	14.1	-	11.2	-	12.7	-	9.6	-	8.9	-	8.1	-	9.8	-	-

* Only values above 75% were taken into account when calculating the average efficacy

Table 3. The efficacy of some combinations of fungicides with insecticides - acaricides in controling of pathogens and pests in the eggplant crop in the field (Vidra, 2017)

Variant	Pathogens and pests														Average efficacy (%)*
	Alternaria solani		*Botryris cinerea*		*Phytophthora parasitica*		*Leptinotarsa decemlineata*		*Macrosiphum euphorbiae*		*Tetranychus urticae*		*Helicoverpa armigera*		
	GA (%)	E (%)	FA (%)	E (%)	FA (%)	E (%)	GA (%)	E (%)	GA (%)	E (%)	GA (%)	E (%)	FA (%)	E (%)	
V1.	1.7	85.0	2.0	79.6	1.3	87.6	1.8	79.3	1.1	85.7	3.9	42.6	3.4	58.5	83.4
V2.	1.9	84.8	2.2	77.5	1.5	85.7	4.2	51.7	3.2	58.4	0.7	89.7	4.0	51.2	84.4
V3.	2.1	83.2	2.3	76.5	1.0	90.5	4.5	48.3	2.8	63.6	4.6	32.3	1.1	86.6	84.2
V4.	2.2	82.4	2.2	77.5	0.8	92.4	1.5	82.7	1.4	81.8	4.2	38.2	3.7	54.9	83.4
V5.	2.5	80.0	2.4	75.5	1.0	90.5	4.6	47.1	3.5	54.5	0.6	91.2	3.9	52.4	84.3
V6.	2.3	81.6	2.4	75.5	1.2	88.6	4.8	44.8	3.0	61.0	4.3	36.8	1.0	87.8	83.4
V7.	2.0	84.0	1.3	86.7	1.8	82.8	1.7	80.4	1.2	84.4	3.7	45.6	3.4	58.5	83.7
V8.	2.3	81.6	1.3	86.7	1.6	84.8	4.4	49.4	3.7	51.9	0.9	86.8	4.1	50.0	85.0
V9.	2.1	83.2	1.5	84.7	2.0	80.9	4.9	43.7	2.9	62.3	4.8	29.4	1.3	84.1	83.2
V10.	12.5	-	9.8	-	10.5	-	8.7	-	7.7	-	6.8	-	8.2	-	-

* Only values above 75% were taken into account when calculating the average efficacy

In 2016, the yield obtained from the eggplant crop in the field were between 33.5 t / ha (V 1) and 43.1 t / ha (V 6), and for the untreated control variant it was 30.4 t / ha (table 4). The highest yields were obtained at variants 6 (43.1 t / ha; 141.8%), 5 (38.9 t / ha; 127.9%) and 4 (38.1 t / ha; 125 ,3 %).

Table 4. Influence of treatments with different combinations of pesticides on the yield of eggplant in the field (Vidra, 2016)

Variant	Yield				Significance
	kg/m^2	t/ha	% compared to the untreated control variant	Difference from untreated control variant (t / ha)	
V1.	3.35	33.5	110.2	+ 3.1	***
V2.	3.43	34.3	112.8	+ 3.9	***
V3.	3.66	36.6	120.4	+ 6.2	***
V4.	3.81	38.1	125.3	+ 7.7	***
V5.	3.89	38.9	127.9	+ 8.5	***
V6.	4.31	43.1	141.8	+ 12.7	***
V7.	3.44	34.4	113.2	+ 4.0	***
V8.	3.55	35.5	116.8	+ 5.1	***
V9.	3.74	37.4	123.0	+ 7.0	***
V10.	3.04	30.4	-	-	-

LSD $_{5\%}$ = 0.771; LSD $_{1\%}$ = 1.051; LSD $_{0.1\%}$ = 1.423

In 2017 the yields obtained from the eggplant crop in the field were between 34.6 t/ ha (V 1) and 44.0 t / ha (V 6), and in the untreated control variant it was 31.1 t / ha (table 5). The highest yields were obtained at variants 6 (44.0 t / ha; 141.5%), 5 (39.8 t / ha; 128.0%) and 4 (38.7 t / ha; 124 .4 %).

Table 5. The influence of treatments with different combinations of pesticides on the yield of eggplant in the field (Vidra, 2017)

Variant	Yield				Significance
	kg/m^2	t/ha	% compared to the untreated control variant	Difference from untreated control variant (t / ha)	
1.	3.46	34.6	111.2	+ 3.5	***
2.	3.48	34.8	111.9	+ 3.7	***
3.	3.74	37.4	120.2	+ 6.3	***
4.	3.87	38.7	124.4	+ 7.6	***
5.	3.98	39.8	128.0	+ 8.7	***
6.	4.40	44.0	141.5	+ 12.9	***
7.	3.52	35.2	113.2	+ 4.1	***
8.	3.61	36.1	116.1	+ 5.0	***
9.	3.82	38.2	122.8	+ 7.1	***
10.	3.11	31.1	-	-	-

LSD $_{5\%}$ = 1.157; LSD $_{1\%}$ = 1.578; LSD $_{0.1\%}$ = 2.136

CONCLUSIONS

- The application of three treatments with the combinations between Acrobat MZ 69 WG, Melody Compact 49 WG or Ortiva Top with Mospilan 20 SG ensured a good protection of the eggplant plants from the attack of harmful organisms like: *Alternaria porri* f.sp. *solani*, *Botrytis cinerea, Phytophthora parasitica, Leptinotarsa decemlineata and Macrosiphum euphorbiae;*
- The combinations between Acrobat MZ 69 WG, Melody Compact 49 WG or Ortiva Top with Vertimec 1.8 EC protected well the eggplant plants from the attack of pathogens of *Alternaria porri* f.sp. *solani,Botrytis cinerea, Phytophthora parasitica* and the pest *Tetranychus urticae*;
- The combinations between Acrobat MZ 69 WG, Melody Compact 49 WG or Ortiva Top with Laser 240 SC ensured a good protection of the eggplant plants from the attack of harmful organisms like *Alternaria porri* f.sp. *solani, Botrytis cinerea, Phytophthora parasitica* and *Helicoverpa armigera;*
- The best results in terms of yield were obtained in variants 6, 5 and 4 (V 6 - Melody Compact 49 WG 0.2% + Laser 240 SC 0.05 - 43.1 t / ha in 2016 and 44 t / ha in 2017; V 5 - Melody Compact 49 WG 0.2% + Vertimec 1.8 EC 0.1% - 38.9 t / ha in 2016 and 39.8 t / ha in 2017; V4 - Melody Compact 49 WG 0.2% + Mospilan 20 SG 0.04% - 38.1 t / ha in 2016 and 38.7 t / ha in 2017).
- In variant 6, the yield had increased from 12.7 t / ha in 2016 to 12.9 t / ha in 2017; in variant 5, the production had increased compared to the untreated control from 8.5 t / ha in 2016 to 8.7 t / ha in 2017 and in variant 4 the yield had increased compared to the untreated control from 7.7 t / ha in 2016 and 7.6 t / ha in 2017;
- We appreciate that the superior yield results obtained for these variants are due especially to the high efficacy of the fungicide Melody Compact 49 WG on the fungus *Phytophthora parasitica* (89.7%, respectively 89.5 on average over the two years).

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FRUIT GROWING

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BREEDING OF NEW PEAR WINTER CULTIVAR 'PANDORA'

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ABSTRACT

Psylla and fire blight resistance combined with fruit quality are still pearbreeding aims at Research Institute for Fruit Growing (RIFG) Piteşti, Romania. The new pear cultivar 'Pandora' was registered in 2019, being released by interspecific hybridization between 'Euras' cv. [(*Pyrusserotina*xOlivier de Serres) x Doyenne d'hiver] and 'Tse Li' cv. (*P.ussuriensis*). The trees are medium vigor, weak branching and semi-upright habit being productive and with low tendency to biennial bearing. The fruit ripens 10-15 days earlier than 'Euras', at end of September.Fruit weight is about 250g, flesh is yellowish white, fine, crisp, juicy, sweetand flavored. The skin color changes from green to yellow upon maturity. It is highly resistant to fire blight and pear psylla under the standard spraying program. Therefore, this new cultivar shows a good potential for commercial fruit growing.

Keywords: breeding, *Pyrus*, resistance, description.

INTRODUCTION

'Pandora' is a new winter cultivar released from the pear breeding program of Research Institute for Fruit Growing (RIFG) Piteşti, Romania. It is a long storage cultivar, very attractive and flavored. It is resistant to fire blight and pear psylla under the standard spraying program.

The pear breeding program started in 1967 at RIFG Piteşti andhad as objectives new cultivars with high quality fruit and resistance to fire blight (*Erwinia amylovora* Burill), tolerance to fumagine (*Capnodium salicinum*) and pear psylla.Twelve dessert pear cultivars have already been released: 'Trivale' (1982), 'Triumf' (1983), 'Argessis' (1985), 'Daciana', 'Carpica' (1989), 'Getica', 'Monica' (1994), 'Ervina' (2003), 'Paramis' (2008), 'Paradise', 'Paradox' (2010), 'Isadora' (2012). 'Monica'is favored for its appearance and 'Daciana' for its taste.

Interspecific hybridization of *Pyrus* has been released by RIFG Piteşti since 1991, when the fire blight disease spread in pear orchards for the first time. The breeding strategy involves the intercrossing of European (*P. communis* Linn.), Japanese (*P. pyrifolia* Nakai) and Chinese (*P. bretschneideri* Rehd.) in order to combine buttery juicy texture with crisp texture, sweet and strong flavor, to extend storage period, to increase the resistance to pests and diseases. The interspecific hybridization is still consider a method with great potentialof pear breeding especially for ripening season extension, fruit quality, disease resistance and cold hardiness (Layne, 1997).

MATERIALS AND METHODS

'Pandora' was obtained by artificial pollination between 'Euras' cv. (registered in 1994, by Research Station for Fruit Growing Voinesti, Romania, authors: NistorAndrieş and Gheorghe

Moruju) and 'Tse Li' cv. (synonym 'Tsu Li' or 'Su Li', an ancient pear cultivar from Shandong, China, introduced at Research Institute for Fruit Growing Piteşti in 1994, from USA,by CociuVasile) made in 2003. The pedigree of interspecific hybridization of 'Pandora' is shown infigure 1. Seedlings were raised in 2004 and planted in selection filed, on own roots, in 2005. The seedlings began to crop in 2011 and the hybrid tree was selected as a promising one in 2013. The new cultivar was registered in 2019 by the State Institute for Variety Testing and Registration with certificate number 9730/24.10.2019, authors: N. Branişte and Mădălina Militaru.

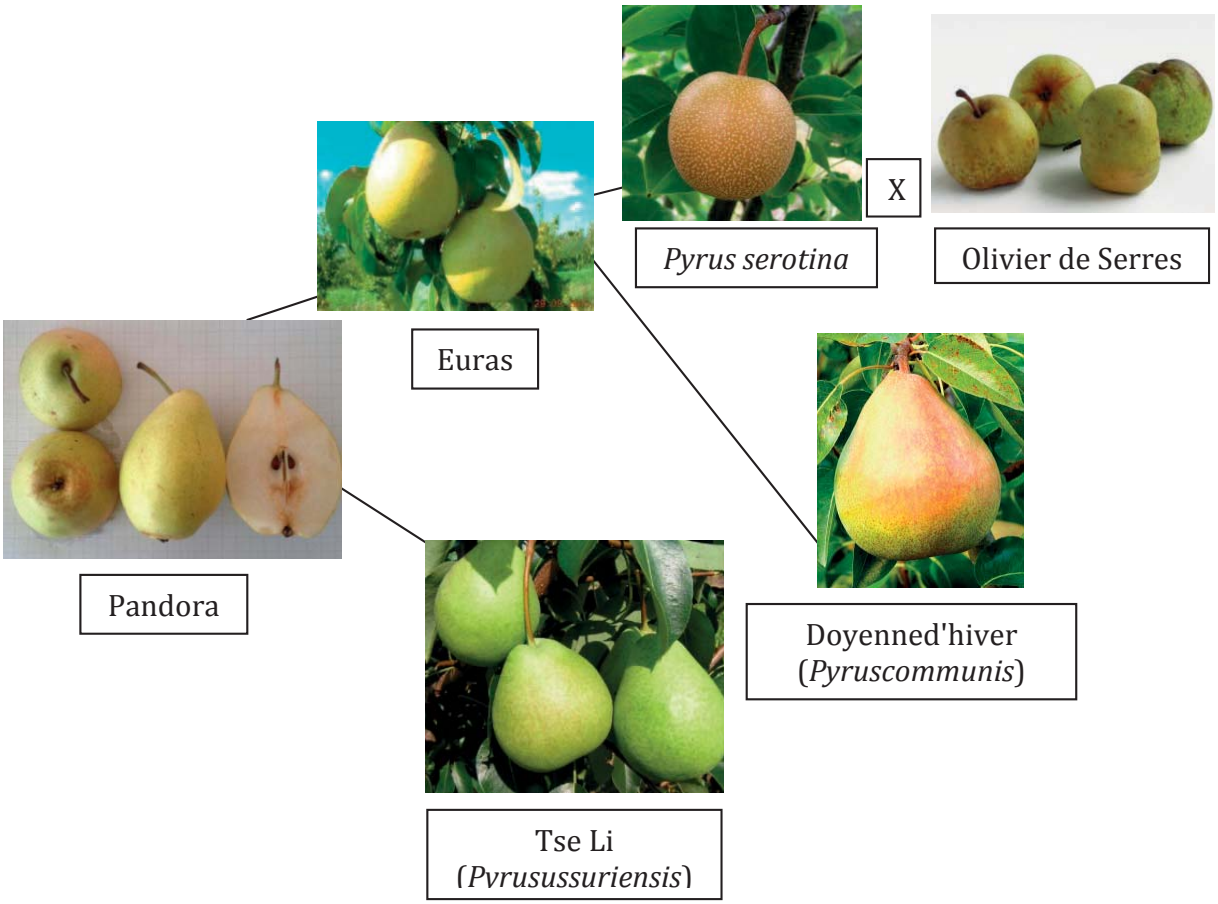

Figure 1. Pedigree of 'Pandora'cv.

RESULTS AND DISCUSSIONS

Tree

The habit is semi-upright with medium vigor. The fruit cropping is generally high and regular every year. Even after a high cropping season, the tendency toward biennial bearing is very low. The vegetative buds are big size and shape of apex is obtuse. The leaf blade is 9.96 cm in length, 5.66 cm in width and 2.5 cm length of petiole. The incisions of margin of leaf blade are bluntly serrate. The corolla is 3.4 cm in diameter and white. Most of flowers have 5 petals and a few flowers have 6-7 petals. The petals are large (15.4 mm in length and 10.5 mm in width) and circular in shape (Table 1, Fig. 2a).

Fruit

'Pandora' fruit is large, about 250 g weight as average (for young trees, 280-300 g/ fruit), 8.7 cm length, 7.3 cm the maximum diameter and ratio length/diameter is 1.2.The fruit stalk is 3.21 cm in length and 2.8 mm in thickness (Table 2, Fig. 2b). At harvest, the

depth andwidth of eye basin are medium, 11.08 mm depth and 26.55 mm width, respectively. The flesh is yellowish white, fine, crisp, juicy, sweet, strongly flavorful and typical aroma with few grit cells. The juiciness varied from juicy to very juicy, depending on the maturity stage. The soluble solids content is 16.8% Brix, higher than 'Euras' cv. Fruits of 'Pandora' has a green ground color at harvest, which changes into yellow after storage.

Flowering and harvest time

Flowering occurs a few days along about a week, before 'Monica' and 'Euras'cvs. Harvest maturity is the same as 'Monica' cv. (end of September in Piteşti, Argeş), but one month earlier than 'Euras' cv. Fruit mature homogeneously within the tree, so that only two pickings are required. 'Pandora' cv. is not susceptible to preharvest drop and the storage ability is excellent, the fruits keepingbeing 120-130 days under cold storage. Fruit picked too early, after long storage, can show some brown core and sometimes flesh browning. The shelf life of 'Pandora' proved to be good: stored until January at 1^0C, the shelf life period was about two weeks.

Table 1. Cultivar description using UPOV guidelines

No. UPOV	Characteristics	States of expression	Note
1	Tree: vigor	medium	5
2	Tree: branching	weak	3
3	Tree: habit	semi-upright	3
4	One year old shoot: growth	zig-zag	3
5	One year old shoot: length of internode	long	7
6	One year old shoot: predominant color on sunny side	brown red	5
7	One year old shoot: number of lenticels	many	7
8	One year old shoot: shape of apex of vegetative bud	obtuse	2
9	One year old shoot: position of vegetative bud in relation to shoot	markedly held out	3
10	One year old shoot: size of bud support	large	7
11	Young shoot: anthocyanin coloration of growing tip (during rapid growth)	weak	3
12	Young shoot: intensity of pubescence (upper third)	strong	7
13	Leaf blade: attitude in relation to shoot	outwards	2
14	Leaf blade: length	long	7
15	Leaf blade: width	medium	5
16	Leaf blade: ratio length/width	large	7
17	Leaf blade: shape of base	obtuse	3
18	Leaf blade: shape apex (excluding pointed tip)	right-angled	2
19	Leaf blade: length of pointed tip	medium	5
20	Leaf blade: incisions of margin (upper half)	bluntly serrate	3

No. UPOV	Characteristics	States of expression	Note
21	Leaf blade: depth of incisions of margin	shallow	3
22	Leaf blade: curvature of longitudinal axis	medium	5
23	Petiole: length	medium	5
24	Petiole: presence of stipule	absent	1
26	Shoot: location of flower bud	mainly on spurs	1
27	Flower bud: length	long	7
28	Flower: sepal length	long	7
29	Flower: attitude of sepals in relation to corolla	adpressed	1
30	Flower: position of margins of petals	overlapping	3
31	Flower: position of stigma in relation to stamens	above	3
32	Flower: size of petal	large	7
33	Flower: shape of petal (excluding the claw)	circular	1
34	Flower: shape of base of petal (excluding the claw)	truncate	3
36	Immature fruit: color of sepals (early summer)	green-brown	2
37	Fruit: length	long	7
38	Fruit: maximum diameter	large	7
39	Fruit: ratio length/diameter	large	7
40	Fruit: position of maximum diameter	clearly towards calyx	3
41	Fruit: size	very large	9
42	Fruit: symmetry (in longitudinal section)	strongly asymmetric	3
43	Fruit: profile of sides	convex	3
44	Fruit: ground color of skin	green	2
45	Fruit: relative area of over color	absent or very small	1
48	Fruit: relative area of russet on cheeks	small	3
49	Fruit: relative area of russet around stalk attachment	absent or very small	1
50	Fruit: length of stalk	medium	5
51	Fruit: thickness of stalk	medium	5
52	Fruit: curvature of stalk	absent or very weak	1
53	Fruit: attitude of stalk in relation to axis of fruit	oblique	2
54	Fruit: depth of stalk cavity	deep	7
55	Fruit: attitude of sepals (at harvest)	converging	1
56	Fruit: eye basin (at harvest)	present	9
57	Fruit: depth of eye basin (at harvest)	medium	5
58	Fruit: width of eye basin (at harvest)	medium	5

No. UPOV	Characteristics	States of expression	Note
59	Fruit: relief of area around eye(at harvest)	smooth	1
60	Fruit: texture of flesh	medium	5
61	Fruit: firmness of flesh	firm	7
62	Fruit: juiciness of flesh	juicy	7
63	Seed: shape	elliptic	3
64	Time of beginning of flowering	very early	1
65	Time of maturity for consumption	late	7

Table 2. Fruit characteristics of 'Pandora' on 2016-2018 at RIFG Pitești

Cultivar	Mean fruit weight (g)	Total soluble solids (Brix, %)	Titrable acidity (%)	pH	Duration of storage (days)	Yield* (kg tree^{-1})
Pandora	250a	16.8a	0.60a	5.24a	125a	16.78a
Euras	156c	14.6b	0.52a	4.92b	140b	13.86b
Monica	175b	12.8c	0.48a	3.94c	106c	15.62a

*Values are the mean for 4 to 6 year old trees, grafted on *Pyruscommunis* seedlings
The different letter indicates significant differences between means according to Duncan's multiple range test, P≤0.05

Pest and disease susceptibility

During 2016-2018, period of observation, no symptoms of fire blight and psylla.

a. b.

Figure 2. 'Pandora' flowers (a) and fruits (b)

CONCLUSIONS

• 'Pandora' produced attractive fruits, with crispy, juicy and tasty flesh, a good storability and a very good shelf life.
• It is licensed in Romania by the State Institute for Variety Testing and Registration and is presently available from RIFG Pitești nursery.

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PHYSICAL AND COMPOSITIONAL CHARACTERISTICS OF CHESTNUT FRUITS

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ABSTRACT

The objective of this study was investigationofphysical and compositional fruits characteristics of six chestnut cultivars. The fruits belonging to these cultivars were subjected to physical and chemical composition determinations, and the obtained results were comparatively analysed. It can be noticed that there are significant differences between cultivars for all the analysed traits. A high variability was found between cultivars in terms of nutrients content. The moisture content of chestnuts analysed in this study ranged from 51.63% to 58.42%. The protein content recorded values between 5.40 and 7.18% fresh matter. The polyphenols content in cultivars taken under analysis was between 1.65 and 19.60 mg GAE/g, which is a very large variation range between different cultivars. The obtained values of antioxidant activity of chestnuts are high, ranging from 0.73 to 9.90 mg Trolox/g of fresh substance, revealing also a wide variation range. Among the individual phenolic compounds, elagic and gallic acids were determined in higher amounts. The results of this study have shown that chestnut fruits contain significant concentrations of primary and secondary metabolites that are known for their positive effects on human health.

Keywords*:* antioxidant capacity; chestnut; flavonoid content; nutritional quality parameters; phenolics

INTRODUCTION

For many centuries, sweet chestnuts (*Castaneasativa* Mill.) have been some of the most important food resources in European rural areas, but the emergence of severe pathologies as well as the depopulation of mountain areas have caused a gradual decline in their consumption (Adua, 1998). Recently, the growing demand for traditional food has increased the interest in this resource and the improvement of its capitalization. The assortment includes hundreds of cultivars with specific characteristics and chemical composition. Knowledge of product composition can offer new opportunities for the chestnut market, since some chestnuts types are suitable for fresh consumption while others are more suitable for drying, producing of flour or pastries (Bounouset al., 2005). Numerous studies have been conducted on nutritional composition of European chestnuts (Bassi et al., 1984; Pinnavaia et al., 1993; Künsch et al., 1999; Tarquini et al., 2001; Attanasio et al., 2004; Sacchetti et al., 2005; Bellini, 2005). Data on cultivars cultivated in Romania are still limited

and incomplete because most of these studies do not report all compositional characteristics. An important aspect in the chemical characterization of chestnuts is that the data in the literature shows great variability, and sometimes it is difficult to describe the chemical characteristics of chestnut cultivars. This is due to several reasons, among which are included: i) some data refers to chestnuts and others refers to marrons, which are products with different morphological characteristics and technological characteristics; ii) some cultivars have different ecotypes with different chemical characteristics (Sacchetti et al., 2005) related to ecological environment; iii) different clones of the same cultivar could exhibit a different chemical composition (Pinnavaia et al., 1993); iv) chestnut composition shows a dramatic variation determined by the harvesting year (Pinnavaia et al., 1993; Sacchetti et al., 2005). In addition, the interaction between years and cultivar is also significant (Ferreira-Cardoso et al., 2005). Another source of variation in data about composition is that some literature references relate about the fresh fruit, while others refers to dried fruit, which represent the bulk of commercial products available on the market.

The aim of this study was to investigate the composition of chestnut fruits from six chestnut cultivars that were grown at R.S.F.G. Valcea. Another important objective of this study was to determine the content of bioactive antioxidant compounds (total polyphenols, individual phenolic compounds, flavonoids) in chestnuts, as well as their in vitro antioxidant activity, aswellas to test the potential use of chestnuts as natural source of antioxidant compounds.

MATERIALS AND METHODS

Materials. Six chestnut cultivars of French origin were studied: 'Marisol', 'Maraval', 'Casval', 'Bournette', 'PrecoceMigoule' and 'Marissard', all cultivated at Valcea Research Station (45°07' N / 24°22' E). The fruits belonging to these cultivars were subjected to physical determinations and chemical composition, and the results obtained were comparatively analysed. Regarding the physical properties of fruits, determinations were made on biometric data (fruit diameters and height), individual mass and fruit volume. Laboratory analyses were conducted on moisture content, total dry substance, titratable acidity, total content of phenolic compounds, total flavonoid content as well as antioxidant activity of fruits. 20 fruits for each cultivar have been individually measured and weighed. After harvesting, the samples were stored at -20°C for one month. To determine the moisture content and titratable acidity, the fruits were stored at +4°C and used the next day.

Determination of nutritional quality parameters (moisture content, total dry matter, titratable acidity). The moisture content was gravimetrically determined by drying the amount of 5 g of fresh vegetable product up to a constant weight in a laboratory oven (Memmert, Germany) that was set at 105°C. Titratable acidity (% citric acid) was measured by titrating an aqueous extract of plant matter with a 0.1 N NaOH solution using phenolphthalein as an indicator.

Determination of total content of phenolic compounds. Total content of phenolic compounds was spectrophotometrically determined by colorimetric method (Singleton et al., 1965) using gallic acid (99% purity, Sigma) as the standard calibration substance. The Folin-Ciocalteu (2N, Merk) reagent and anhydrous sodium carbonate (99% purity, Sigma) were also used. Samples (3 g of plant product) were extracted with 5 mL of methanol in a BandelinSonorex Digital 10P ultrasonic bath for 45 min at ambient temperature. After extraction, the samples were centrifuged for 5 min at 4200 rpm and the supernatants were filtered through polyamide membranes with 0.45 μm holes. To 100 μL of each methanolic extract, 5 mL of distilled water and 500 μL of Folin-Ciocalteu reagent were added. After at least 30 seconds and maximum 8 minutes, 1.5 mL of sodium carbonate solution (20% w / v)

was added. The reaction mixture was diluted with distilled water for a final volume of 10 mL. The same procedure was applied to standard gallic acid solutions. The absorbance at 765 nm of each mixture was measured with a Varian Cary 50 UV spectrophotometer (Varian Co. USA) after incubation for 30 min at 40°C. The results were expressed in mg of gallic acid equivalent (GAE)/100 g.

Determination of total flavonoid content. Determination of flavonoids was performed using the aluminium nitrate colorimetric method described by Mohammadzadeh et al. (2007). Briefly, 0.5 mL of extract was diluted with (1:10) methanol and mixed with 0.1 mL of 10% aluminium nitrate, 0.1 mL of aqueous solution of 1 M potassium acetate and 4.3 mL of methanol. After storing them for 40 minutes at room temperature, the absorbance of the mixture was determined at 415 nm. The mixture of reagents without the sample extract was used as control. Quercetin was used to prepare the standard curve (0-100 mg/l). The samples were analysed in triplicate and the results were expressed in milligrams equivalent of quercetin/100 g (mg QE/100 g).

Determination of antioxidant capacity. Antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method described by Oliveira et al. (2008), with some modifications. Extraction of samples was carried out according to the same protocol described in the determination of total polyphenol content. Each methanolic plant extract (50 µL) was mixed with 3 mL of methanolic solution containing 0.004% (v/v) DPPH. The mixture was vigorously stirred and allowed to stand at room temperature and in darkness for 30 min, after which the absorbance decrease was measured at 517 nm using the Varian Cary 50 UV-Vis spectrophotometer. Capture capacity of DPPH free radical was then calculated using the formula: DPPH capture capacity (%) = [1 – Abssample/Abscontrol] × 100, where Abscontrol is the absorbance of control (DPPH solution without sample) and Abssample is the absorbance of the sample to be analysed. The capture capacity of DPPH free radical was then expressed in relation to Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which was used as standard reference. The following substances and reagents were used: methanol (Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Merck). The DPPH radical was freshly prepared and protected from light. A methanol/water mixture served as control and was run in each test. All analyses were performed in triplicate. The results were expressed in mmol of Trolox/100 g.

Determination of individual phenolic compounds by HPLC. High Performance Liquid Chromatography analyses were performed using a Surveyor Thermo Electron HPLC system comprising a Surveyor Plus LCPMPP pump, a Surveyor Plus ASP autosampler, a PDA 5P array diode detector, and the Chrom Quest 4.2 manager system as data processing system, using the method described by Nour et al. (2013). Separation was carried out in reverse phase on a Hypersil Gold C18 column (particle size 5 mm, 250 mm x 4.6 mm) supplied by Thermo Electron Corporation (San Jose, CA). The mobile phase was made up of a solution of 1% acetic acid (A) and methanol (B). Samples were eluted using the following gradient: 90% A from 0 to 27 min, from 90 to 60% A in 28 min, 60% A for 5 min, from 60 to 56% A in 2 min, 56% A for 8 min, from 56 to 90% A in 1 min and 90% A in 4 min to restore the initial conditions, before the next sample is injected. All gradients were linear. The flow rate was 1 mL/min, the injection volume was 5 µL and the column temperature was maintained at 20°C. Chromatograms were recorded at three wavelengths (254, 278 and 300 nm) corresponding to the maximum absorption of the analysed compounds. Each compound was identified by retention time and by standard enrichment under the same conditions. The identity of constituents was also confirmed using the diode array detector by comparison with the UV spectra of standards within the wavelength range 220-450 nm. Quantification was performed by external calibration using a five-point curve obtained by dilutions of

standard solutions. Each standard solution was injected into the HPLC system and the calibration curves were plotted by plotting the peak areas according to respective concentrations for each compound. The content of phenolic compounds investigated in the extracts was expressed in mg/100 g as the mean ± standard deviation. The standards of phenolic acids (gallic, vanilic, chlorogenic, caffeic, siringic, p-coumaric, ferulic, synapic, salicylic, elagic and trans-cinnamic) and flavonoids (catechin, epicatechin, routine, miricetinandquercetin) were purchased from Sigma-Aldrich (Darmstadt, Germany). Methanol and acetic acid were purchased from Merck. The water used in the experiments was treated in a SGWater purification system (Merck KGaA, Darmstadt, Germany).

Statistical analysis. Statistical analysis was performed using Statgraphic Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA). The data were presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSIONS

Determinations of physical characteristics of the analysed cultivars reveal, in terms of fruit size, 'Maraval', 'PrecoceMigoule' and 'Marsol' cultivars. All these varieties were also the ones that presented the highest values of fruits mass and volume. 'Bournette' cultivar is worth nothing, because, although having small size fruits, exhibited the highest specific weight among the cultivars analysed. It can be noticed that there are significant differences between cultivars for all the analysed physical traits. The results are shown in Table 1 and Table 2.

Table 1. Diameters, height, shape index and size index determined in analyzed chestnut cultivars

Cultivars	Large diameter (D), mm	Small diameter (d), mm	Height (h), mm	Shape index	Size index
Marsol	35.21±1.69^{cd}	21.23±1.41^{b}	28.9±1.27^{b}	1.01±0.05^{a}	28.28±0.99^{b}
Maraval	39.18±2.24^{e}	23.65±3.68^{c}	33.91±1.32^{d}	1.08±0.07^{bc}	32.25±1.95^{d}
Casval	32.13±1.69^{b}	17.38±1.89^{a}	25.88±1.35^{a}	1.05±0.08^{ab}	25.13±1.26^{a}
Bournette	30.06±2.33^{a}	17.12±2.41^{a}	26.67±1.38^{a}	1.14±0.07^{d}	24.62±1.62^{a}
PrecoceMigoule	36.34±1.64^{d}	20.34±2.09^{b}	31.04±1.16^{c}	1.10±0.05^{cd}	29.24±1.23^{c}
Marissard	34.14±2.32^{c}	18.59±2.62^{a}	31.41±1.51^{c}	1.20±0.10^{e}	28.05±1.74^{b}

*Values in the same column followed by different letters as the exponent are significantly different at $p < 0.05$

Table 2. Mass, volume and specific weight determined in chestnut cultivars varieties analyzed

Cultivars	Mass (g)	Volume ($cm^{3)}$	Specific weight (g/cm^3)
Marsol	10.13±1.04^{d}	13.80±0.94^{c}	0.73±0.03^{bc}
Maraval	12.76±2.20^{e}	15.64±1.21^{d}	0.81±0.08^{cd}
Casval	5.48±0.77^{a}	9.86±0.65^{b}	0.55±0.04^{a}
Bournette	6.64±0.96^{b}	7.64±0.58^{a}	0.87±0.06^{d}
PrecoceMigoule	10.35±1.38^{d}	13.42±1.02^{c}	0.77±0.04^{bcd}
Marissard	8.72±1.86^{c}	12.62±0.87^{c}	0.69±0.10^{b}

*Values in the same column followed by different letters as the exponent are significantly different at $p < 0.05$

The moisture content of chestnuts analysed in this study ranged from 51.63% to 58.42%. In the scientific literature, the values of fresh chestnut humidity were between 41 and 59% (Desmaison et al., 1986; Salvini et al., 1998; De La MontañaMiguelez et al., 2004) with a very high variation coefficient (up to 20%) within the very same cultivar (De La MontañaMiguelezet al., 2004). This fact is due to the chestnut epicarp which is porous and non-lignified, which results in a quick drying of chestnuts compared to other nuts (Bounous

et al., 2005). A series of studies show results of analyses conducted on chestnuts treated in cold water or hot water, therefore some of the variations may be attributed to the influence of these treatments (Sacchetti et al., 2005; Künsch et al., 2001; Jermini et al., 2006).

Nutritional composition of different chestnut cultivars is presented in Table 3. Means and standard deviations are reported for fresh substance. As regards the humidity variation coefficient, it can sometimes be very high (11%), as was reported, for example, by De La MontañaMiguelez et al. (2004) and Sacchettiet al. (2005) in fresh and treated chestnuts, respectively. Regarding the glucidic fraction, it should be noted that sucrose is the main reducing glucid in chestnuts, although small amounts of fructose and glucose traces have also been reported (Desmaison et al., 1986; Sacchetti et al., 2005; Pinnavaia et al., 1993), while maltose was reported only by Attanasio et al. (2004).

Table 3. Chemical composition of fruits in analyzed cultivars

Cultivars	Water (%)	Titrable acidity (g malic acid/100 g)	Lipids (%)	Proteines (%)
Marsol	55.38±1.22^{bc}	0.402±0.03^{b}	0.53±0.03^{c}	5.40±0.33^{a}
Maraval	51.63±1.56^{a}	0.603±0.04^{c}	0.65±0.04^{d}	5.74±0.41^{ab}
Casval	58.42±1.38^{d}	0.670±0.04^{d}	0.35±0.02^{a}	5.90±0.38^{ab}
Bournette	53.81±0.98^{ab}	0.234±0.02^{a}	0.41±0.03^{b}	7.18±0.48^{d}
PrecoceMigoule	57.07±1.33^{cd}	0.268±0.03^{a}	0.64±0.04^{d}	6.34±0.39^{bc}
Marissard	54.77±1.67^{bc}	0.402±0.03^{b}	0.53±0.03^{c}	7.02±0.44^{cd}

*Values in the same column followed by different letters as the exponent are significantly different at $p < 0.05$

In the study reported by Neri et al. (2010), sucrose was the representative sugar in all analysed samples, with mean values between 12.95 and 19.84%, values that are consistent with those reported by Senter et al. (1994), Künsch et al. (1999, 2001) and Sacchettiet al. (2005) for ecotypes in Italy, and by De La MontañaMiguelez et al. (2004) for Spanish cultivars. Higher sugars contents (approximately 29-30%) were reported by Attanasio et al. (2004) for chestnuts in Montella, and by Desmaisonet al. (1986) for other Italian ecotypes. Variability of sucrose content may be the result of climatic factors influence and genetic variability. For example, Pinnavaia et al. (1993) reported a high variability of sucrose content (10.45-15.71% d.w.) within the same chestnut ecotype, due both to different years of harvesting and to different clones within the same ecotype. A high variability of sucrose content has also been reported by De La MontañaMiguelez et al. (2004).

Among the different sugars determined by different authors, fructose presented, within the same cultivar, the highest year-to-year variation coefficient (De La MontañaMiguelezet al., 2004; Neri et al., 2010). The protein content recorded values between 5.40 and 7.18% fresh matter. These values are higher than those reported by Pinnavaia et al. (1993) and Sacchettiet al. (2005) for some of the Italian ecotypes. Also, these values are higher than those reported by Kunsch et al. (1999) for some of the Italian and Swiss cultivars, but they are similar to those reported by Ferreira-Cardoso et al. (1993, 2005) in some of the Portuguese cultivars and by De La Montana Miguelez et al. (2004); Pereira-Lorenzo et al. (2006) and Peña-Méndez et al. (2008) in Spanish chestnut cultivars. These authors consider that there is a correlation between chestnut protein content and the type of origin soil; for example, chestnuts in shale areas have a much higher protein content than those grown on granite soils (Gomes et al., 1997). Also, many authors have reported that this protein content showed a very large variation coefficient between different years of analysis (Sacchettiet al., 2005).

Ferreira-Cardoso et al. (2005) also reported that the harvest year and interaction between harvest year and cultivar have significantly influenced chestnut protein content. The highest levels of protein content were determined for 'Bournette' and 'Marissard'

cultivars. The lipid content of chestnuts was found to be between 0.35 and 0.65% f.w., much lower than the data reported by Neri et al. (2010),Bassiet al. (1984),Kunsch et al. (1999),Salvini et al. (1998) and Sacchettiet al. (2005) for Italian chestnuts, but closer to those reported by Senter et al. (1994), De La Montana Miguelez et al. (2004) and Borges et al. (2006) for the Italian, Spanish and Portuguese cultivars, respectively.

The polyphenols content in cultivars taken under analysis (Table 4) was between 1.65 and 19.60 mg GAE/g, which is a very large variation range between different cultivars. Antioxidant activity of chestnuts was determined using a method widely used in the literature and expressed in mg Trolox equivalents per gram. Values obtained are high, ranging from 0.73 to 9.90 mg Trolox/g of fresh substance, revealing also a wide variation range. The highest content of phenolic compounds was determined in 'Marissard' cultivar, followed by 'PrecoceMigoule' cultivar. In direct correlation with the phenolic compounds content, the antioxidant activity has also recorded the highest values in the above-mentioned cultivars.

Table 4. Antioxidant activity, total content of phenolic compounds and total flavonoid content in the fruits of chestnut cultivars

Cultivar	Total content of phenolic compounds (mg GAE/g)	Total content of flavonoids (mg QE/g)	Antioxidant activity (mg Trolox/g)
Marsol	2.79 ± 0.18^{b}	1.80 ± 0.21^{b}	1.78 ± 0.09^{c}
Maraval	1.88 ± 0.14^{a}	2.63 ± 0.17^{c}	1.30 ± 0.11^{b}
Casval	1.65 ± 0.23^{a}	0.84 ± 0.09^{a}	0.73 ± 0.03^{a}
Bournette	2.15 ± 0.22^{ab}	1.79 ± 0.12^{b}	1.55 ± 0.16^{bc}
PrecoceMigoule	12.12 ± 0.68^{c}	8.57 ± 0.56^{d}	6.96 ± 0.36^{d}
Marissard	19.60 ± 0.78^{d}	14.77 ± 0.66^{e}	9.90 ± 0.48^{e}

*Values in the same row followed by different letters as the exponent are significantly different at $p < 0.05$

In general, in case of chestnuts, this high antioxidant activity is only partially based on phenolic compounds, considering that the high content of ascorbic acid in chestnuts has an important contribution to antioxidant activity (Proteggenteet al., 2002). This fact is important due to low thermal stability of ascorbic acid which could cause a dramatic decrease in the antioxidant activity of chestnuts as a result of their roasting.

Values of total phenolic compound content are close to those reported by De Vasconcelos et al.(2007), who found values between 15.8 and 22.7 mg GAE/g in chestnuts of Portuguese cultivars 'Martainha', 'Longal' and 'Judia', while other studies reported a content of 147 mg/g dry matter, which corresponds to about 54 mg/g of fresh substance (Callisteet al., 2005).

Among the individual phenolic compounds (Table 5), elagic and gallic acids were determined in higher amounts.

De Vasconcelos et al. (2007) have also determined large amounts of free gallic acid in Portuguese chestnut cultivars, the highest content recorded being 9.1 mg/g of fresh substance. Also, free elagic acid has been determined in chestnut fruits as well as many elagitinins and procyanidins. De Vasconcelos et al. (2007) reported 9.6, 2.7, and 4.8 mg/g fresh substance in chestnuts of 'Martainha', 'Longal', and 'Judia', respectively. There is strong evidence on beneficial health effects of gallic and elagic acids, with regard to their antioxidant activities, and their positive effects on cardiovascular functions and anticancer activity.

Table 5. Content of phenolic compounds (flavonoids and phenolic acids) (mg/100 g) in chestnuts

Genotypes	Marval	Bournette	PrecoceMigoule	Marsol	Marissard	Casval
Gallic acid	53.48± 0.14	88.5±0.14	193.61± 0.14	58.37±0.23	303.43±0.38	33.62±0.27
Catechinhydrate	33.5±0.27	36.98±0.27	223.52±0.15	24.69±0.27	241.62±0.15	20.72± 0.14
Vanilic acid	7.56± 0.09	27.42±0.22	51.46±0.15	25.04±0.17	8.11±0.08	6.49±0.24
Caffeic acid	nd	nd	7.65±0.08	7.17±0.19	nd	4.52±0.07
Syringic acid	5.46±0.08	9.17±0.15	52.23±0.27	5.32± 0.09	76.24±0.34	17.43±0.25
Epicatechin	10.03± 0.56	0	186.83± 0.56	32.33±0.21	137.606±0.56	10.22±0.22
Coumaric acid	4.32± 0.09	5.11± 0.14	19.13±0.15	9.93±0.08	140.84±0.27	16.57± 0.14
Ferulic Acid	3.28±0.12	3.21±0.08	20.35±0.19	8.68±0.24	124.95±0.35	3.89±0.08
Synapic Acid	5.73± 0.14[a]	5.35± 0.09	38.16±0.17	17.05± 0.14	269.01±0.34	11.67±0.16
Salicilic Acid	11.75±0.15	20.48±0.05	47.49±0.15	19.21±0.07	106.67±0.25	3.71± 0.09
Rutin	14.58±0.23	17.94±0.56	29.36±0.0.655	27.27±0.23	161.41± 0.14	11.78±0.08
Elagic acid	20.07±0.27	28±0.09	251.62± 0.56	36.14±0.27	238.79±0.48	18.76±0.27
Myricetin	2.44±0.08	1.68±0.05	24.24±0.15	3.47±0.15	8.72±0.21	3.29±0.22

CONCLUSIONS

Fruits of six chestnut cultivars of French origin that are grown at Valcea Research Station were characterized with regard to their nutritional and antioxidant composition. A high variability was found between cultivars in terms of nutrients content, a low lipid content but a high protein content.

All cultivars have exhibited a high content of phenolic compounds and high antioxidant activity, these results indicating a potential use of chestnuts as a source of natural antioxidant compounds in a diet.

The results of this study have shown that chestnut fruits contain significant concentrations of primary and secondary metabolites that are known for their positive effects on human health. Chestnuts are an increasingly popular food, equally consumed fresh, frozen or baked, or processed in various ways (marron glacé, pastries).

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PRELIMINARY RESULTS REGARDING THE BEHAVIOUR OF SOME NEW APRICOT CULTIVARS IN BUCUREȘTI AREA

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ABSTRACT

The study presents the behaviour of 28 apricot cultivars planted in 2017 in the Experimental Field of the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The foreign cultivars grafted on Myrobalan 29C, Saint Julien A and GF 677, were planted at 4.5 x 1.5 m and 3.5 x 2.0 m for Parallel U and respectively, 3.5 x 2.0 m for Trident canopy. Trees vegetative growth was evaluated by analysing the trunk cross section, tree height, fruiting branches number and length. First fruit yield was heighted and the productivity index was calculated. The results show differences in growth correlated with the canopy, cultivars and used rootstocks. Correlations between analysed parameters are presented. As a conclusion, the differences between the two canopies, Parallel U and Trident, using different types of rootstocks show their influence on the annual vegetative growth and on apricot trees productivity. Some preliminary conclusions can be drawn regarding the advantages and disadvantages of using the two canopies in apricot orchards.

Key words: *Armeniaca vulgaris, rootstocks, canopies, correlations.*

INTRODUCTION

Apricot is an important fruit crop for the southern and western regions of Romania with a long tradition of cultivation (Bălan et al., 2008). Several breeding programs are active in Europe (Asma, 2012; Audergon et al., 2012; Auvinet et al., 2020; Bassi and Foschi, 2020; Bălan, et al., 2008; Egea et al., 2012;Krška and Vachůn, 2016; Nesheva et al., 2020; Milatović et al., 2012; Opriţa et al., 2020; Suran and Skřivanová, 2020; Topor et al., 2010) and worldwide (Dejampour, 2012; Liu et al., 2012; Gouble et al., 2020; Xue et al., 2020) so, there is a constant interest to promote and introduce new cultivars. In order to evaluate the behaviour of new genotypes in different cultivation area (Gouble et al., 2020), field trials are essential and the results over few testing years need to be used when the introduction of a new cultivar is decided (Stănică et al., 2010; Stănică and Eremia, 2014; Tabakov and Yordanov, 2012; Zaman et al., 2018). In fruit trees, the field performances of a new cultivar, regarding the tree vigour, growth, yield and fruit quality, besides its genetic heritage, are essentially influenced by the rootstock (Duval et al., 2012; Krška et al., 2012; Tabakov and Yordanov, 2012), planting system and tree canopy (Stănică et al., 2012; Matei et al., 2013; Stănică and Eremia, 2014; Negru, 2019; Negru and Peșteanu, 2019). Besides the traditional Open Vase canopy, used in classical low-density plantings, in the modern commercial apricot orchards, canopies with one, two or three vertical axes are more diffused (Meland, 2001; Musacchi, 2008; Stănică, 2019. The vertical axe canopies have some important

advantages (Robinson et al., 2011; Dorigoni et al., 2011; Stănică, 2019), being closer to the natural tree growth tendency (Lauri et al., 2011), easy to conduct and maintain, and giving the possibility of annual renewal of the fruit shoots (Neri et al., 2010).
The present paper presents some preliminary results regarding the behaviour of 28 cultivars, most of them new introduced to Romania, cultivated in Bucharest on two canopies Parallel U and Trident on different rootstocks.

MATERIALS AND METHODS

In 2017, at the Experimental Field of the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest, 28 apricot cultivars have been planted. Most of the cultivars were newly introduced to Romania: Congat, Primando, Primaya, Rubista, Wonder Cot, Lady Cot, Delice, Lily Cot, Milord, Swired, Mikado, Lido, Med Flo, Flopria, Faralia, Farely, Farbali, Fartoli, Farbela, Anegat, Farlis, Farclo, while others: The Best of Hungary, Bergeron, Vitillo, Boccuccia Liscia, Portici, Pisana, are already tested and known. The apricot cultivars were grafted on three rootstocks: GF677, Mirobolan 29 and Saint Julien A. Two canopies and two planting systems were tested: Parallel U, planted at 4.5 x 1.5 m and 3.5 x 2.0 m and Trident, planted at 3.5 x 2.0 m. Trees were conducted on a four wires trellis system with 4.5 m high concrete poles. On the row, drip irrigation was provided by a drip line with 0.5 m between self-compensated (2 l/hour) drippers. Between the rows, the soil was kept grass-covered with regular mowing. Tree vigour was evaluated by measuring tree height and axis length, trunk cross section (TCS) and the total annual shoots vegetative growth.
In the same time, tree productivity was expressed by the yield expressed on kg/tree and t/ha, and by the productivity index – kg/cm^2 TCS.

Rubista Lido Primaya

Figure 1. Some of the tested apricot cultivars: Rubista, Lido and Primaya

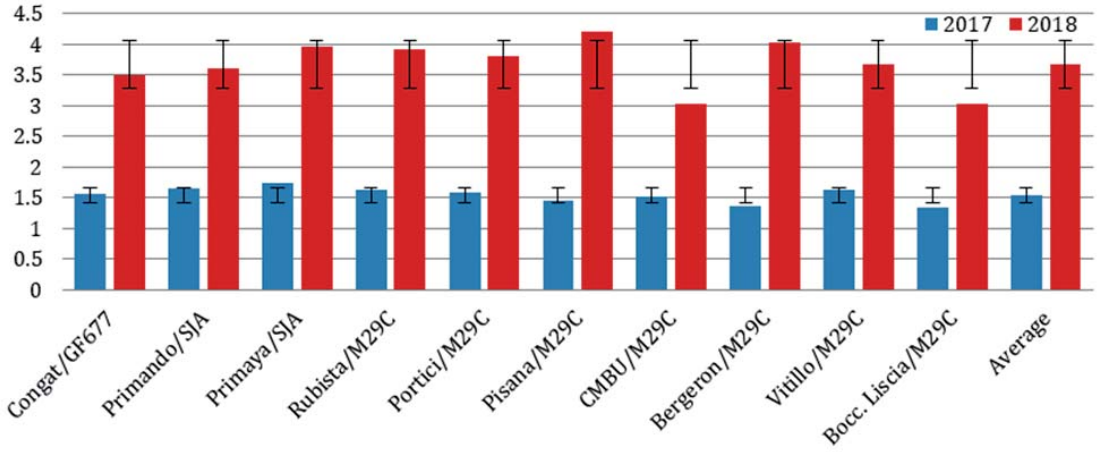
RESULTS AND DISCUSSIONS

1. Tree vigour

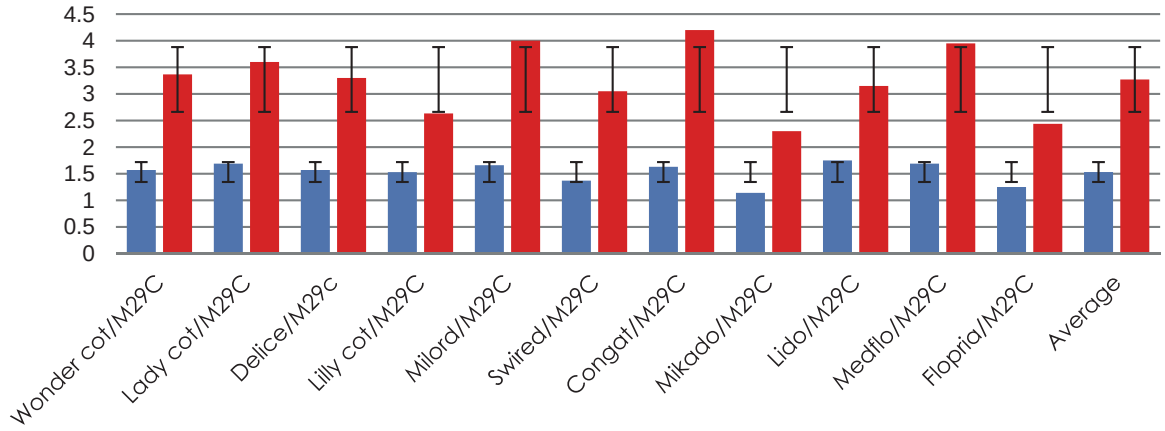
Tree height was both influenced by cultivar and rootstock. As one can see in the Figure 2 (a and b), in the first year after planting, most of the cultivars over passed 1.5 m in height after the pruning back of the initial shoot at 50 cm. Lower growth, under 1.5 m in height, was registered at Mikado/M29C, Flopria/M29C, Bergeron/M29C and Boccuccia Liscia/M29C.

After the second growing season, the most vigorous cultivars were Pisana/M29C and Congat/M29 C, followed by Bergeron, Milord and Medflo, also grafted on Myrobalan 29 C.

GF 677 induced a lower growth on Congat than Myrobalan 29C.



a



b

* Vertical bars are indicating the standard deviation (SD)

Figure 2 (a, b). Apricot tree height influenced by cultivar and rootstock – Parallel U canopy

After the first growing season, the trees conducted as Trident registered an average height of 1.6 m (Figure 3). The most vigorous cultivars were Farbali/Saint Julien A, followed by Farclo/SJA, with over 1.75 m height. The lowest vigour, less that 1.5 m height, was measured at Farely and Primaya, both grafted on Saint Julien A.

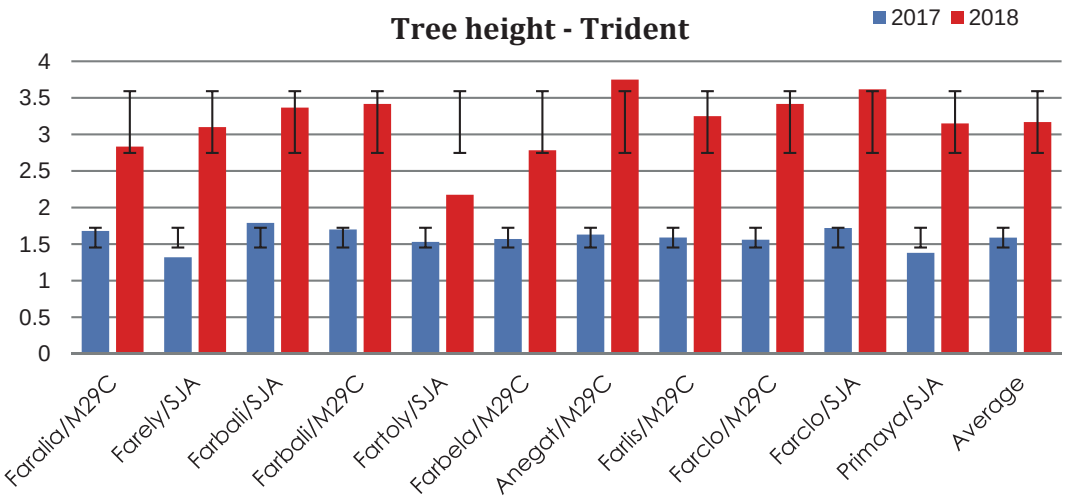
At the end of the second growing season, the most vigorous cultivars, that over passed 3.5 m in height, were Anegat/M29C and Farclo/SJA. The lowest growth was measured at Fartoly/SJA, Farbela/M29C and Faralia/M29C.

Do to the fact that, at Trident canopy, each tree formed three axes the vigour was distributed in three, the average tree vigour was lower than at Parallel U, were the growth vigour was distributed on two axes.

Trunk cross section was also influenced by cultivar, rootstock and canopy, as it is reflected by the Figure 4 a and b and Figure 5.

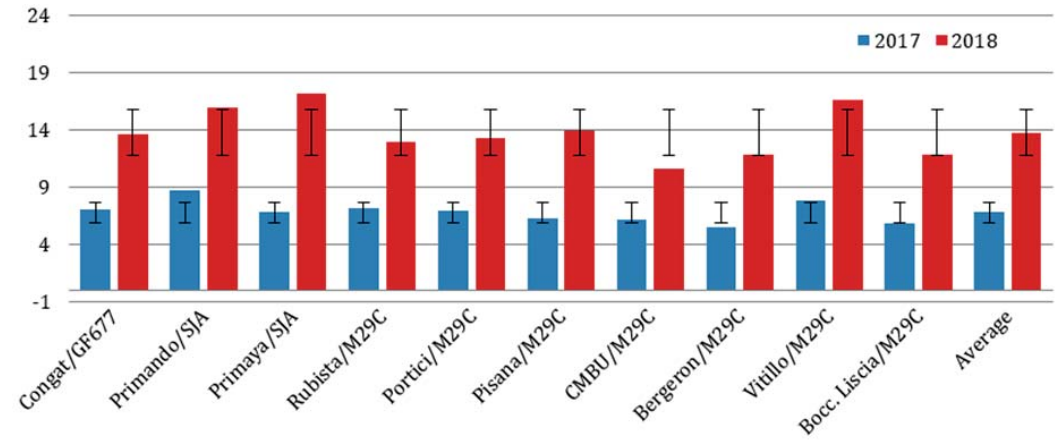
At the end of the first growing year, the trunk cross section varied, for Parallel U canopy, from 2.51 cm^2 at Mikado/M29C, the less vigorous cultivar to Lido (11.16 cm^2) and Milord (11.05 cm^2), the most vigorous cultivars, both grafted on Myrobalan 29C.

In average, during the second growing season, the TCS had a spectacular growth for all cultivars.



* Vertical bars are indicating the standard deviation (SD)

Figure 3. Apricot tree height influenced by cultivar and rootstock – Trident canopy



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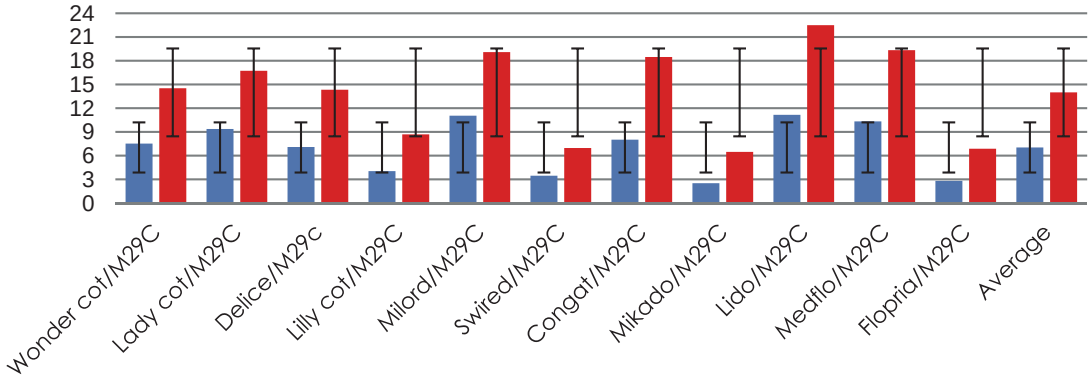


b

* Vertical bars are indicating the standard deviation (SD)

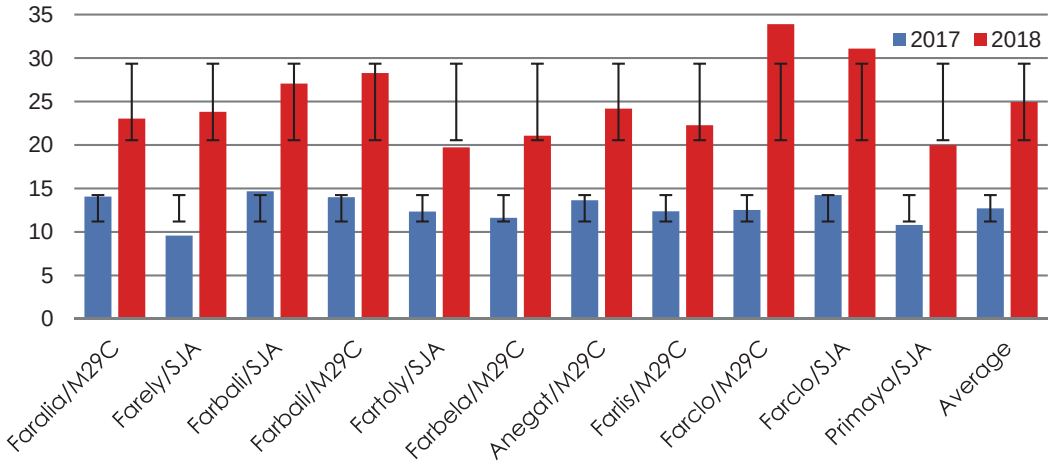
Figure 4 (a, b). Apricot trunk cross section (cm²) influenced by cultivar and rootstock Parallel U

The highest TCS value was registered at Lido grafted on M29C (22.49 cm²), followed by Medflo (19.53 cm²) and Milord (19.09 cm²), both grafted on Myrobalan 29C. The smallest

TCS, was instead measured at Mikado (6.47 cm²) followed by Flopria (6.87 cm²), both grafted on Myrobalan 29C.

The trunk cross section (TCS) at Trident canopy registered higher values at all cultivars and cultivars with an average of 12.72 cm², in the first growing year and 24.95 cm², in the second growing year.

The highest TCS value was registered by Farclo, grafted on M29C (33.90 cm²) and the same cultivar, grafted on SJA (31.09 cm²). The smallest TCS value was measured at Fartoly/SJA (19.74 cm²), followed by Primaya/SJA (19.99 cm²).



* Vertical bars are indicating the standard deviation (SD)

Figure 5. Apricot trunk cross section (cm²) influenced by cultivar and rootstock Trident

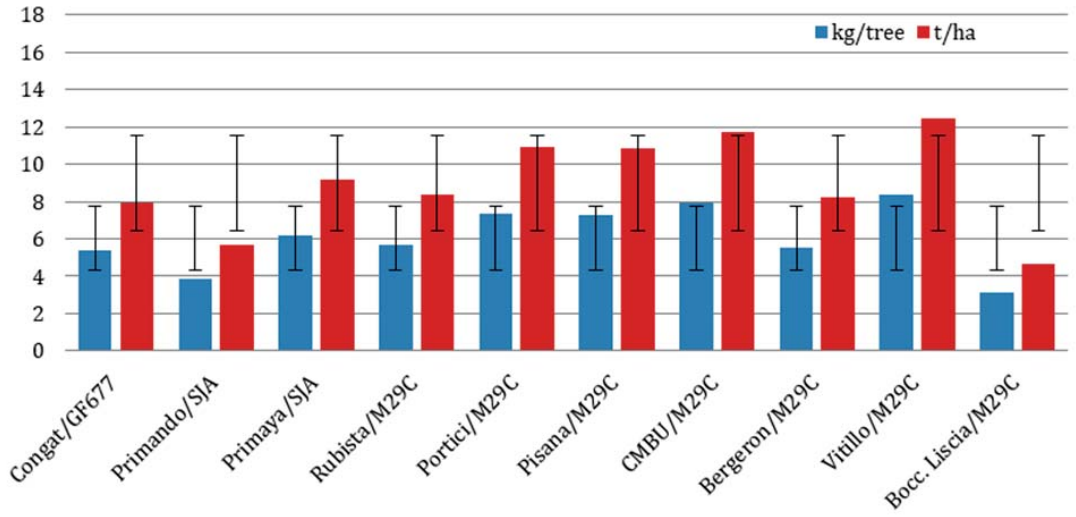
By analysing the correlation existing between the trunk cross section and tree height, we found out in the first growing year a high correlation index that varied from 0.77 for Parallel U (4.5 x 1.5 m), 0.91 for Parallel U (3.5 x 2.0 m) to 0.97 for Trident (3.5 x 2.0 m). In the second year, the correlation decreased and regression equations are presented in Table 1.

Table 1. Correlation between Trunk Cross Section and Tree Height

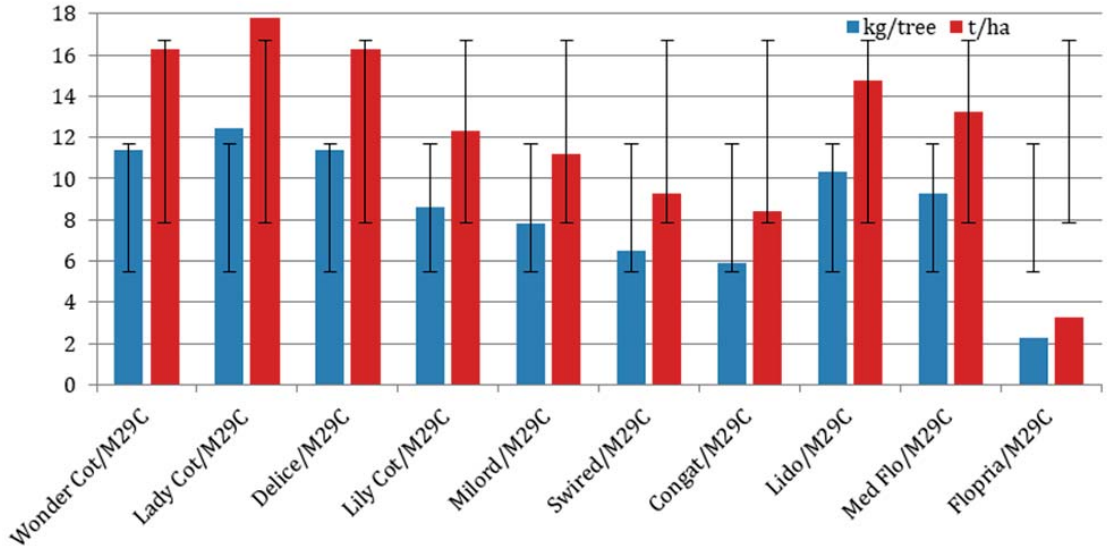
Canopy and planting distances	Year	R	R^2	Regression equations
Parallel U (4.5 x 1.5 m)	2017	0.77	0.57	y = 0.1012 x + 0.859
	2018	0.42	0.18	ns
Parallel U (3.5 x 2.0 m)	2017	0.91	0.83	y = 0.0539 x + 1.1526
	2018	0.80	0.63	y = 0.0872 x +2.0512
Trident (3.5 x 2.0 m)	2017	0.97	0.94	y = 0.086 x + 0.4944
	2018	0.64	0.41	y = 0.0613 x +1.6401

x = Trunk cross section; y = Tree height

2. Tree productivity, expressed as kg/tree and respectively, t/ha, was influenced by the genotype, rootstock and canopy (Figure 6 a and b and Figure7).



a



b

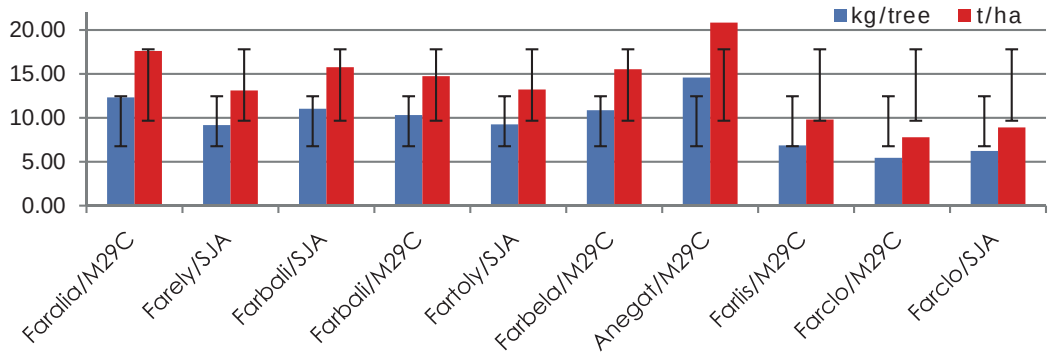
* Vertical bars are indicating the standard deviation (SD)

Figure 6 (a, b). Apricot productivity (kg/tree and t/ha) influenced by cultivar and rootstock Parallel U canopy

When analysing the results of Parallel U canopy, the most productive in the second year after planting, was the cultivar Lady Cot (17.75 t/ha), followed by Wonder Cot (16.25 t/ha) and Delice (16.24 t/ha), all cultivars being grafted on Myrobalan 29C. The lowest yield was registred at Flopria with only 3.25 t/ha and Boccuccia Liscia (4.68 t/ha).

The cultivars conducted as Trident registered a higher yield comparing with Parallel U, Anegat being on the first place (20.85 t/ha), followed by Faralia (17.62 t/ha). Lowest yield was produced by Farclo (7.79 t/ha).

The productivity index (not presented in this paper) expressed as kg/cm^2 TCS, varied a lot with the cultivar between 0.16 (Farclo/M29C) and 0.91 (Lily Cot/M29C).



* Vertical bars are indicating the standard deviation (SD)

Figure 7. Apricot productivity (kg/tree and t/ha) influenced by cultivar and rootstock - Trident

CONCLUSIONS

The new tested apricot cultivars showed in general a good adaptation in the first two years of vegetation, by realising a strong growth and reaching the planned height, with a slight delay at Trident canopy, were the tree vigour was split between the three axes.

There were registered important differences in vigour between studied cultivars while, generally, Myrobalan 29C induced a superior vigour.

Even at the end of the first growing year, the trunk cross section(TCS)was quite similar, in the second year, each cultivar started to expressed its vigour. In the first growing year strong correlation was found between TCS and tree height.

Most of the studied cultivars expressed their precocity and high productivity and produced, in the second year after planting, a top yield of 20.85 t/ha (Anegat/M29C). Under Trident, the first yield was higher, probably because of a denser canopy permanent structure realized by the three axes, in comparison to only two axes in Parallel U.

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YIELD AND FRUIT QUALITY OF SOME PLUM CULTIVARS IN ECOLOGICAL SYSTEM

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ABSTRACT

The aim of this study was to assess the yield and fruits quality of plum produced in ecological system.In 2019-2020 periods the influence of different fertilizers (Biohumus, Macys BC 28 and Cifamin BK) on yield and fruits quality at three plum cultivars ('Centenar', 'Tita' and 'Stanley') was carried out in a demonstrative plot from Research Institute for Fruit Growing Pitești, Romania. Biohumus was applied to the soil in increasing dosesfrom 0.5 l/tree (respectively 415 l/ha), 0.7 l/tree (respectively 585 l/ha) to 0.9 l / tree (respectively 750 l/ha) in two moments: in spring before the start of vegetation and in autumn after the fall of the leaves.Macys BC 28 and Cifamin BK fertilizers were applied foliar in doses of 2 l/ha, respectively 1 l/ha, in two moments: after flowering and in the young fruit phase.As results of the investigations we found that: the highest fruit yield was obtained at 'Centenar' (47.36 kg/tree) and 'Stanley' (41.00 kg/tree) cultivars in fertilization variant 3 (Biohumus – 0,9 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application);the best results regarding the fruits weight were also obtained in the 3rd fertilization variant (Biohumus – 0,9 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application), among the varieties being noted the 'Tita'cv. with an average fruit weight of 59. 14 g; the fruits soluble solid content was higher in the case of the fertilized variants than in the unfertilized variant and the fruits acidity was higher in the case of the unfertilized variant than in the fertilized variants.

Keywords: plum, cultivar, fertilizers, yield, fruits quality.

INTRODUCTION

In the last decade, demand for ecological products by European consumer's increased (Amarante et al., 2008, Cuevas et al., 2015).
Consumers have started to look for safer and better controlled foods produced in more environmentally friendly. Ecological produced foods are widely believed to satisfy the above demands, leading to lower environmental impacts and higher nutritive values.
European Union guidelines' regarding ecological management practices exclude the use of synthetic pesticides and fertilizers, allowing the use of animal and green manures, compost, sulfur and copper products, botanical insecticides, traps and other biological control methods (Holb et al., 2003, Peck et al., 2006, Jonsson, 2007, Amarante et al., 2008).
Ecological plum production is still quite limited in most countries (including Romania), due mainly to the inadequate control of pests, diseases and weeds with organic alternatives. Regarding fertilization, in ecological system we can use compost, green fertilizers, manures

or other fertilizers certified for biological application (Yadav et al., 2000, Hristova et al., 2017).
Ecological agriculture has the potential to reduce the impacts of agriculture on humans and ecosystems, but it has been claimed that organic production system are less efficient, fruits production being lower than in the conventional production system (McArtney and Walker, 2004;Talamini do Amarante et al., 2008; Malezieux et al., 2018).
The major reason to choose organically grown fruits, besides the concern for environmental issues, is the improvement of fruits quality. Several articles show that ecological fruits have a higher content in micronutrients, phenolic compounds, vitamins etc. (Young et al., 2005, Raigon et al., 2010, Cuevas et al., 2015).
The aim of this paper was to evaluate yield and some important quality parameters of three plum cultivars from orchards managed under ecological system.

MATERIALS AND METHODS

The experimental field was established in 2009at RIFG Piteşti – Mărăcineni. Three plum cultivars grafted on 'Myrobalan C5' rootstock were planted in a spacing of 4 m between the rows and 3 m between trees, according to the following experimental scheme: Factor A – cultivar, with three graduation (a1-'Centenar',a2-'Tita' and a3-'Stanley'); Factor B – fertilization variant, with four graduations (b1-Biohumus – 0,5 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application; b2 - Biohumus – 0,7 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application; b3 – Biohumus – 0,9 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application; b4 – 'Unfertilized').
Biohumus is a 100% organic fertilizer, produced with the help of earthworms, which stimulates the yield, growth and health of trees.In the plum demonstration plot, soil fertilization with Biohumuswas carried out in increasing doses, from 0.5 l/tree (respectively 415 l/ha), 0.7 l/tree (respectively 585 l/ha) to 0.9 l / tree (respectively 750 l/ha) in 2019 – 2020 in two moments: in spring before the start of vegetation and in autumn after the fall of the leaves.
Macys BC 28 is a fertilizer based100% on the algae*Macrocystisintegrifolia*, which stimulates root development, vegetative growth, flowering and fruiting, in also the fruits size and quality.Macys BC 28 fertilizer was applied foliar in 2019 – 2020 in doses of 2l/ha, in two moments: after flowering and in the young fruit phase.
Cifamin BK is a special fertilizer based also on the algae *Macrocystisintegrifolia*, very rich in organic components, indicated for improving the size and fruits quality, keeping fruit and firmness unaltered, ensuring optimal shelf-life.Cifamin BKfertilizer was also applied foliar in 2019 – 2020 in doses of 1 l/ha, also in two moments: after flowering and in the young fruit phase.
Foliar fertilizers, Macys BC 28 and Cifamin BK were dissolved in 500 l water.
The experiment was carried out in a randomized block design, in 3 replications with 3 trees per plot.
In 2019 - 2020 periods, the following measurements were carried out: fruits yield in kg/tree; mean fruits weight in g; soluble solids content of fruits with a digital refractometer in % Brix; malic acidcontent of fruitsin % or g/100 g fresh matter with the device Minititrator Hanna Instrument 84532; fruits firmness was measured with non-destructive penetrometer Qualitest HPE equipped with a plunger of diameter 0.10 cm.
The results of the experiment were analyzed statistically using Duncan's multiple range test at a 0.05% significance level.

RESULTS AND DISCUSSIONS

The influence of factor A (cultivar) on fruits yield

Regarding the fruitsyield, in kg/tree, it can be observed that, on average, on the 4 fertilization variants, the 'Centenar'cv. registered the highest fruit production (39.20 kg/tree) significantly exceeding the 'Stanley' and 'Tita'cvs. (with 6.68 kg/tree in the case of 'Stanley' cv. and with 10.48 kg/tree in case of 'Tita' cv.). Also, fruit yield on the 'Stanley'cv. compared to the 'Tita'cv. was significantly higher by 3.8 kg/tree (Table 1).

The influence of factor B(fertilization variants) on fruits yield

On average, on the three cultivars studied, between the fertilized and unfertilized variants there are significant differences.The fruits production per tree increased with increasing Biohumus doses (from 31.82 kg/tree in V1 to 39.58 kg/tree in V3). Thus, the fertilization variant 3 determined a higher fruit production than the other variants, respectively 39.58 kg/tree, exceeding the fruits production obtained in V1 with 7.76 kg/tree, with 7.62 kg/tree inV2 and with 9.03 kg/tree in unfertilized variant (Table 1).

In conclusion, the highest fruits production was obtained on 'Centenar' (39.58 kg/tree) and 'Stanley'cvs. in fertilization variant 3 - Biohumus – 0,9 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application.

Even if it is not the subject of this paper, in order to see if the ecological fertilization is efficient, the fruit production obtained at the three varieties in ecological system was compared with the one obtained in conventional system. We find that yields in ecological systems were on average 10 to 20% lower than those in conventional orchard, results obtained by other authors like McArtney and Walker (2004), Amarante et al. (2008), Malezieux et al. (2018).

Table 1. Influence of the fertilizers on the yield (kg/tree)

No.	Cultivar	Fertilization variant				
		V1	V2	V3	V4	Average
1	Centenar	37.05	37.31	47.36	35.07	**39.20 a**
2	Tita	29.87	27.69	30.39	26.94	**28.72 c**
3	Stanley	28.56	30.89	41.00	29.63	**32.52 b**
	Average	**31.82 b**	**31.96 b**	**39.58 a**	**30.55 b**	

Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P>0.05).

The influence of factor A (cultivar) on fruitsweight

The highest value of fruits weight was recorded onthe 'Tita'cv., which significantly exceeded the average fruit weight of the 'Centenar'cv. by 20 g and the 'Stanley' cv. by 18.84 g (Table 2).

The influence of factor B (fertilization variants) on fruitsweight

The fruit weight was influenced by the fertilization variants at all cultivars studied. It can be seen from Table 2 that in the four different formula fertilization treatments, variant 3 has the best effect, and the average fruit weight per fruit is the largest reaching 48.96 g on average, being very significant differences between V3 and unfertilized variant.

The highest fruitsweight was obtained on 'Tita' cv. (64.47 g) in fertilization variant 3 - Biohumus – 0,9 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + CifaminBK – 1 l/ha, foliar application (Table 2).

Table 2. Influence of the fertilizers on the fruits weight (g)

No.	Cultivar	Fertilization variant				
		V1	V2	V3	V4	Average
1	Centenar	39.53	38.53	40.67	37.70	**39.11 b**
2	Tita	57.23	60.97	64.47	53.90	**59.14 a**
3	Stanley	38.67	42.20	41.73	38.60	**40.30 b**
	Average	**45.14 bc**	**47.23 ab**	**48.96 a**	**43.40 c**	

Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P>0.05).

The influence of factor A (cultivar) on fruitssoluble solids content

A more significant difference was found in fruits soluble solids content. The results obtained are ranged from 16.15% Brix at 'Tita' cv. and 12.22% Brix at 'Stanley' cv., between cultivars being significantly difference (Table 3).

The influence of factor B (fertilization variants) on fruits soluble solids content

The three different fertilization variants have a greater influence on the fruits quality of the all plum cultivars studied, compared with unfertilized variant.

The highest values were found in all three fertilization variants (over 14% Brix), while in the unfertilized variant the content of fruits in soluble solids content was much lower (12.68% Brix).

In case of this trait, the best results were obtained on 'Tita' cv. in fertilization variant 1 - Biohumus – 0,5 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application.

Table 3. Influence of the fertilizers on the fruits soluble solids content (% Brix)

No.	Cultivar	Fertilization variant				
		V1	V2	V3	V4	Average
1	Centenar	13.27	14.54	14.60	12.37	**13.70 b**
2	Tita	17.27	17.23	15.63	14.47	**16.15 a**
3	Stanley	12.49	11.52	13.67	11.20	**12.22 c**
	Average	**14.34 a**	**14.43 a**	**14.63 a**	**12.68 b**	

Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P>0.05).

The influence of factor A (cultivar) on content of fruits in malic acid

The highest content of fruits in malic acid was recorded 'Tita'cv. (0.53 g/100 g fresh matter), which significantly exceeded the 'Centenar'cv. by 0.17 g/100 g fresh matter and the 'Stanley' cv. by 0.24 g/100 g fresh matter (Table 4).

The influence of factor B (fertilization variants) on content of fruits in malic acid

The content of fruits in malic acid ranged from 0.47% in unfertilized variant to 0.36%in variant 2 (Biohumus – 0,7 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application). In all fertilization variants the fruits acidity was lower than in unfertilized variant (Table 4). The same results obtained Hristova et al. (2017) at plum cultivar 'Tegera'. Amarante et al. (2008) came to the conclusion that apple fruits from organic orchard had lower titratable acidity than fruit from conventional orchard.

The highest malic acid content was recorded on 'Tita' cv. in fertilization variant 1 - Biohumus – 0,5 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application.

Table 4. Influence of the fertilizers on the content of fruits in malic acid (g/100 g fresh matter)

No.	Cultivar	Fertilization variant				
		V1	V2	V3	V4	Average
1	Centenar	0.27	0.34	0.29	0.54	**0.36 b**
2	Tita	0.63	0.47	0.55	0.49	**0.53 a**
3	Stanley	0.26	0.27	0.26	0.39	**0.29 c**
	Average	**0.39 b**	**0.36 b**	**0.37 b**	**0.47 a**	

Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P>0.05).

The influence of factor A - cultivar on fruitsfirmness

Stanley' cv. had firmer fruits than 'Tita' cv. in all the fertilization variants, between these two cultivars being very significant differences.

The influence of factor B - fertilization variants on fruitsfirmness

The fruits of all cultivars studied had higher flesh firmness at harvest time in all fertilization variants than unfertilized variant (Table 5), results confirmed by other authors as well DeEll and Prange (1992), Reganold et al. (2001), Weibel et al. (2004), Peck et al. (2006) at apple. A significant difference is found between fertilization variant 1 and unfertilized variant (60.14 HPE units in V1 and 54.01 HPE units in V4) (Table 5). '

The 'Tita' cv. in fertilization variant 1 - Biohumus – 0,5 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + CifaminBK – 1 l/ha, foliar application, had the highest value of fruits firmness.

Table 5. Influence of the fertilizers on the fruits firmness (HPE units)

No.	Cultivar	Fertilization variant				
		V1	V2	V3	V4	Average
1	Centenar	62.22	62.53	54.68	61.07	**60.12 a**
2	Tita	51.22	48.88	53.68	48.68	**50.61 b**
3	Stanley	66.98	62.40	66.74	52.28	**62.10 a**
	Average	**60.14 a**	**57.94 ab**	**58.37 ab**	**54.01 b**	

Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P>0.05).

There arevery significant correlations between: fruit weight and soluble solids content (Fig. 2); fruit weight and malic acid content; firmness and fruit weight (Fig. 3). There aredistinctly significant correlations between: yield and fruit weight (Fig. 1); fruit soluble solids content and firmness; fruit firmness and acid malic content; fruit soluble solids content and malic acid content. We find also significant correlations between: yield and fruit firmness; fruit weight and firmness; content of fruit in malic acid and yield (Table 6).

Table 6. Correlation coefficients r obtained between parameters studied

r	Yield (kg/tree)	Fruit weight (g)	SU (% Brix)	Firmness (units HPE)	Malic acid (g/100 g fresh matter)
Yield (kg/tree)	x	-0.4630**	-0.1233	0.3829*	-0.2766
Fruit weight (g)	-0.4441**	x	0.7824***	-0.4107*	0.7175***
SU (% Brix)	-0.0632	0.7228***	x	-0.4418**	0.5067**
Firmness (units HPE)	0.2510	-0.5996***	-0.4418**	x	-0.5066**
Malic acid (g/100 g fresh matter)	-0.4129*	0.6504***	0.5067**	-0.5066**	x

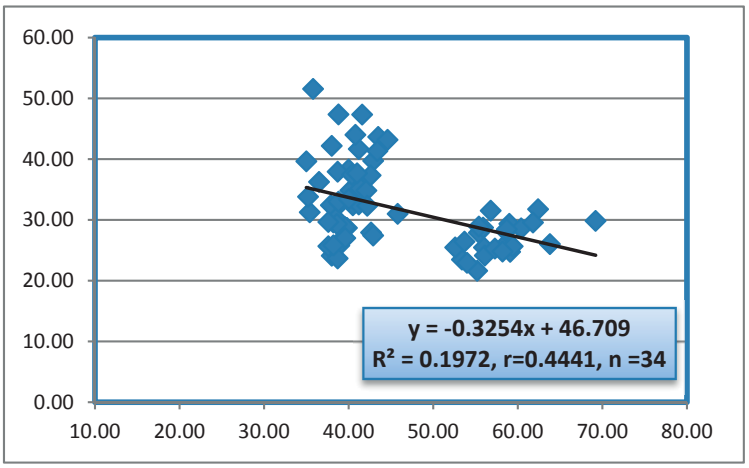

Figure 1. Significant correlation between fruit weight (g) and yield (kg/tree)

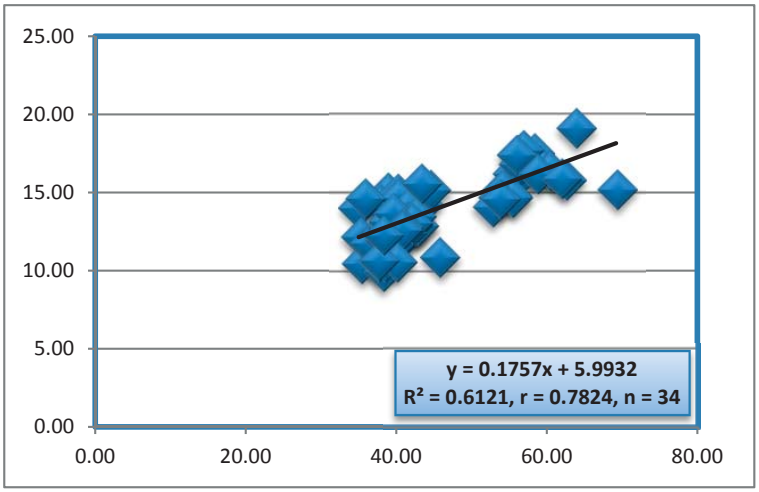

Figure 2. Significant correlation between fruit weight (g) and fruit soluble solids content (% Brix)

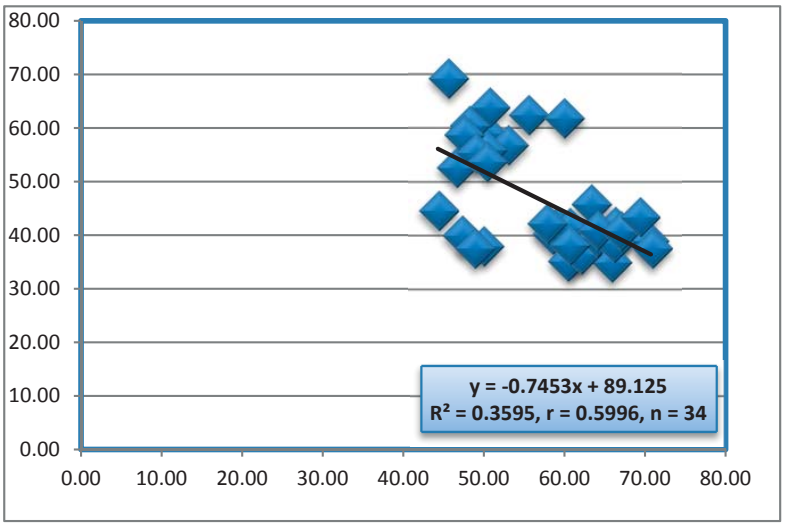

Figure 3. Significant correlation between firmness (HPE units) and fruit weight (g)

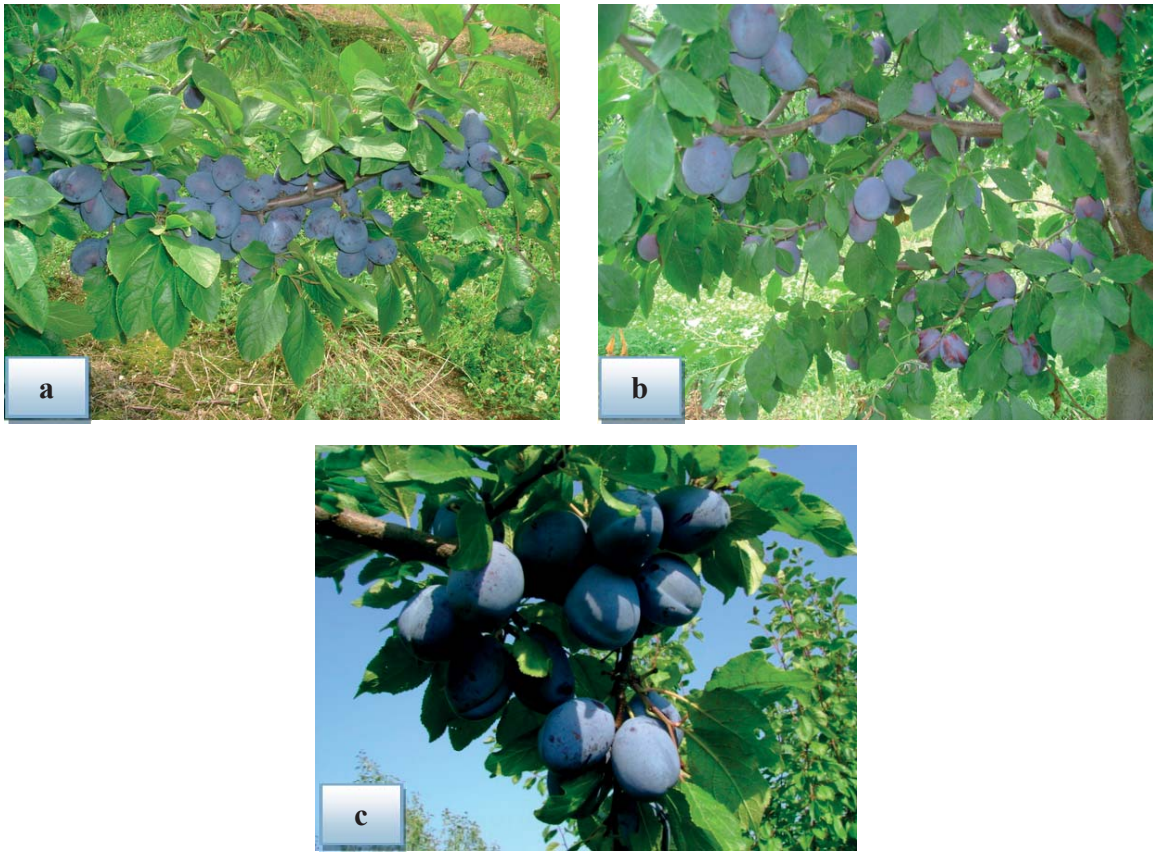

Figure 4. The fruit yield (kg/tree) of plum cultivars (a – 'Centenar', b – 'Tita', c – 'Stanley'') in fertilization variant 3 (Biohumus – 0,9 l/tree, soil application + Macys BC 28 – 2 l/ha, foliar application + Cifamin BK – 1 l/ha, foliar application)

CONCLUSIONS

- The major reason to choose organically grown fruits, besides the concern for environmental issues, is the improvement of fruits quality.
- As results of the investigations we found that:
- the highest fruits yield was obtained at 'Centenar' (47.36 kg/tree) and 'Stanley' (41.00 kg/tree) cultivars in fertilization variant 3 (Biohumus – 0,9 l/tree, ground application + Macys BC 28 – 2 l/ha, foliar application + Cyfamin BK – 1 l/ha, foliar application);
- The best results regarding the fruits weight were also obtained in the 3rd fertilization variant (Biohumus – 0,9 l/tree, ground application + Macys BC 28 – 2 l/ha, foliar application + Cyfamin BK – 1 l/ha, foliar application), among the varieties being noted the 'Tita' cv. with an average fruit weight of 59. 14 g;
- The fruits soluble solid content was higher in the case of the fertilized variants than in the unfertilized variant;
- The fruits acidity was higher in the case of the unfertilized variant than in the fertilized variants.
- The fruits of all cultivars studied had higher flesh firmness at harvest time in all fertilization variants than unfertilized variant.

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EVALUATION OF NEW APPLE CULTIVARS GROWN IN ROMANIAN NORTHEASTERN AREA

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ABSTRACT

The paper presents a 2-years study of the valuable characteristics in four foreign apple cultivars grown in northeastern Romania, named Moldavia area. The phenological stages, quality traits of fruits and some chemical parameters were evaluated. Start flowering was determined at April 17th ('Resse'), while end of flowering was identified the period April 27th -28th. Period of flowering was 9 to 12 days as average for studied years 2019-2020. Fruit's weight have varied between 120.55 g ('Resse') and 179.46 g ('Remo'), while fruit's equatorial diameter have varied between 65.82 mm and 74.87 mm at 'Resse' and respectively 'Remo', but no statistically distinct differences registered between all studied apple cultivars. The values of the soluble dry solids range between 14.65° Brix ('Resse') to 17.05° Brix ('Relinda'), and the total dry solids has recorded values between 17.95% ('Resse') to 21.39% ('Remo'). The studied apple cultivars showed variability but some got remarked through large fruit's size or high level of soluble dry solids.

INTRODUCTION

Apple tree growing is one of the most known and widespread crops in the temperate climates of the globe. The variability of the genus and the main species of *Malus domestica*, favors dissemination and adaptation to various geographical areas (Grădinariu, 2002). Worldwide, approximately 5 million hectares are occupied by apple cultivation providing a total production of 84 million tonnes (FAO, 2020). The share of apple cultivation in the world economy of fruit production is mainly due to the role that fruits have in the rational human diet. They are consumed fresh or processed as alcoholic or non-alcoholic beverages (juices or nectars). In Romania, applestree growing occupies the leading place in fruit production. According to statistical data of the Ministry of Agriculture and Rural Development of Romania, the area cultivated with this species is about 54 thousand hectares, while the total production of apples in Romania can reach 644 thousand tons (Brodeală et al., 2020). For apple growers, fruit size is strongly correlated with profits, but shape and color are also some of the most important qualities for fruit consumers (Schotzko, 1985). Zajmi et al. (2007) show that in practice there are several methods for determining the maturity of the fruit according to market requirements, both for consumers and the processing sector. In practice, the following methods are mainly used: the number of days from pollination to fruit harvest, the separation of the branch stalk, the change of fruit color, the change of seed color, etc. There are also methods of physiological analysis, such as the spectrophotometric method or the determination of the presence of starch (Lepaja K. et al., 2014). According to Shqahu (2007), apple is a culture that grow well on a wide range of soils, both in texture and soil type. It has moderate demands on temperature, giving good results in areas where the average annual temperature is between 8 and 9.5° C, but can

grow and develop in regions with average temperatures of 7.5-7.9° C. The studied apple cultivars showed high variability but some got remarked through large fruit's size or high level of soluble dry substance.

MATERIALS AND METHODS

The research was performed between 2019-2020, at Research and Delopment Station for Fruit Growing (RSFG) Iaşi, using as research material four apple cultivars: 'Golden Reinders', originating from Netherland (Militaru et al., 2018) and 'Remo', 'Relinda' and 'Resse', originating from Germany (Hanke et al.,2017), grafted on MM106. The experimental field is located in the Sârca valley, where the annual average temperature was 11.33°C in 2019 and 12.03°C in the first seven months of 2020 while the multiannual average being 11.68°C. The trees were planted at 2 x 4 m distances and led under the shape ofpalmette. On the tree rows, the soil was prepared with the rotary orchard tiller and between the rows, the soil was grassed. The control of diseases and pests was performed according to the received warnings, phytosanitary treatments being applied. To appreciate the flowering and fructification phenophases the Fleckinger system was used (1960). Biometric measurements and determinations were performed as follows:fruit's equatorial diameter(mm), thicknes (mm) and lenght (mm) using slide gauge tool Lumytools (Radu et al., 1957) and its average weight (g) using an analytical balance Radwag. The chemical determinations included for the analysis: the soluble dry solids (SDS%) using a Zeiss refractometer,the determination of the humidity (%) and of the total dry substance (TDS%) using the oven for five hours to 105°C (Cociu, 1989). The experimental data was interpreted statistically by analysing the variance.

RESULTS AND DISCUSSIONS

The main fructification phenophases of studied apple cultivars are presented in table 1. Bud burst start on 4thof April at 'Remo' cv.,but were end on 6th of April at 'Relinda' cv. Flowering beginning was between 17th to 19th of April, and blooming ending was between 27th to 28th of April. Bloom period of studied apple cultivars was between 9 to 12 days.

Table 1. The main fructification phenophases of studied apple cultivars (RSFG Iaşi, 2019-2020)

Cultivars	Bud burst (data)	Flowering beginning (data)	Flowering ending (data)	Duration period (days)	Ripening time (data)
Golden Reinders	April 5	April 18	April 28	11	September 9
Remo	April 4	April 19	April 27	9	September 4
Relinda	April 6	April 19	April 28	10	September 8
Resse	April 5	April 17	April 28	12	September 4

In order to establish the adaptability of the studied cultivars to the ecological conditions in Iaşi-Romania, biometric determinations as: weight, diameter and height of the fruits were recorded. Thus, were made centralized averages of the obtained values (Table 2). Through the statistical interpretation of the results compared to the average as control, were obtained insignificant differences for all four cultivars. But we finded that the greatest fruit's weight registered 'Remo' and 'Relinda' cvs.with 179.46 g and 168.46 g, respectively.

Regarding the weight of the fruit, the largest differences from the control variant were recorded at 'Resse'cv., with a lower value by 33.5 g (Table 2). However, Zadravec et al. (2013) showed that fruit diameter and fruit weightare positively correlated.

The fruit's lenght and thickness of the studied four apple cultivars did not have significant differences compared with average as control (Table 2).

Table 2. Physical features of the fruit in the investigated apple cultivars
(RSFG Iaşi, 2019-2020)

Cultivar	Fruit's weight(g)	Fruit's equatorial diameter(mm)	Fruit's thickness(mm)	Fruit's length(mm)
Golden Reinders	147.67 ns	71.33 ns	70.03 ns	63.20 ns
Resse	120.55 ns	65.82 ns	63.37 ns	56.02 ns
Remo	179.46 ns	74.87 ns	72.84 ns	66.26 ns
Relinda	168.46 ns	73.45 ns	69.88 ns	65.83 ns
Average (control)	154.03	71.37	69.03	62.83
DL 5%	97.13	14.06	13.05	13.74
DL 1%	141.28	20.46	18.98	19.98
DL 0.1%	211.93	30.68	28.48	29.98

Figure. 1. Relindaapplecv.
(photo Iulia Mineata)

Figure 2. Remoapplecv.
(photo Iulia Mineata)

The average soluble dry solids of the studied four apple cultivars was between 14.65% ('Resse') and 17.05% ('Relinda'). By statistically interpreting distinctly significant differences were recorded for 'Relinda'cv. and negative distinctly significant differences for 'Resse'cv. There were no significant differences in the 'Golden Reinders' and 'Remo'cvs. compared with average as control (Table 3).

Table 3. Chemical characteristics of the fruits in the investigated apple cultivars
(RSFG Iaşi, 2019-2020)

Cultivar	SDS(%)	TDS(%)	Humidity(%)
Golden Reinders	15.90ns	19.01ns	80.98ns
Resse	14.6500	18.96ns	81.03ns
Remo	15.68ns	21.39**	78.6100
Relinda	17.05**	17.950	82.04*
Average (control)	15.82	19.33	80.66
DL 5%	0.64	1.53	1.53
DL 1%	0.93	2.22	2.22
DL 0.1%	1.41	3.33	3.33

The analyses performed to determine the humidity and total dry solids had insignificant differences of 'Golden Reinders' and 'Resse'cvs. compared to the average as

control. The difference from the average of total dry solids in 'Remo'cv. is distinctly significant, while 'Relinda' variety shows negatively significant differences. The humidity of the fruit's showed significant differences in 'Relinda'cv. and negative distinctly significant differences in 'Remo'cv. However, our results are according with other similar research on apple genotypes. Also, Campeanu et al., (2009) find that TDS recording values between 11.63% to 21.31%, while SDS recording values between 11.00% to 15.50%at ten apple genotypes in Romanian climate conditions.

CONCLUSIONS

- The research shows results that have an impact on the technology of apple growing, both for fresh consumation and for processing, by using foreign cultivars of apple in the climatic region of North-East Romania, the Iaşi area.
- All the quality parameters observed on the fruits, such as the diameter, the height, the weight and the content of the soluble dry substance demonstrate that 'Golden Reinders', 'Resse', 'Remo' and 'Relinda' cvs. have a good adaptability in the ecological conditions in Iaşi, keeping the characteristics specific to the cultivar.

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GRAPE POMANCE GENERATION FROM GRAPE CULTIVARS CULTIVATED IN TÂRNAVE VINEYARDS IN THE FRAMEWORK OF THE CLIMATE CHANGE

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ABSTRACT

Grape pomace is a by-product obtained from the technological processing of grapes and represents on average 20% of the total amount of grapes taken for winemaking. Due to the medical, food and cosmetic interest in the valorisation of grape pomace, the present study presents the percentage of pomace resulting, the yield of must and the sugar content of must obtained for 25 grapevine cultivars, hybrids and clones for white and red wines, cultivated in the SCDVV Blaj vineyards from Blaj, Crăciunelu de Jos and Ciumbrud. In the climatic conditions of the year 2020 with heavy rains in June and cold nights in September, the highest amounts of GP are obtained from the white cultivar Hibernal (GP yield33.43% and sugar concentration in must 205 g/L) and from the white cultivar Pinot gris 18-5 (GP yield34.09% and sugar concentration in must 210 g/L). The lowest percentage of GP was obtained in the case of Pinot gris 34 Bl clone, (GP yield18.09% and sugar concentration in must 236 g/L). Our data show that the harvesting time and the terroir influence the GP yield.

Keywords: grape pomace, yield, climat changes, must, Transylvanian grapes

INTRODUCTION

Grape pomace (GP) is a byproduct generated during the winemaking process and it accounts for approximately 10–30% of the mass of grapes crushed (Tomaz et al., 2016; Voşloban et al., 2020). GP is constituted of skins, pulp, stalks and seeds, which account to 25–35 kg per 100 L of produced wine (Mendes et al., 2013). The yield of pomace production varies due to the variability in freshness and moisture contents among the various sources (Muhlack et al., 2017), to the cultivar and terroir, respectively (Voşloban et al., 2020).
GP's generation during the the winemaking process is different for white and red grapes. Thus, seeds and skins are removed before fermentation in white grape winemaking, but not removed only after a maceration period in contact with fermenting must in red grape winemaking (Antoniolli et al., 2015; Tomaz et al., 2016).
Red grapes are entirely involved in fermentation and processed skins contain much less pulp and residual sugars than the skins from white grapes that are mechanically pressed to produce juice and are not subjected to ethanolic fermentation (Silva, 2003; Ruberto et al., 2007; Mendes et al., 2013). Yield is indicator that shows the percentage ratio between the total mass used and the amount of resulting must (Balteş, 2016; Visan et al., 2018), and GP, respectively.The meteorological conditions in which specific grape cultivars are cultivated influence the maturity stage of the grapes and, obviously, the quality of the wine and GP.

Climate change has left its mark on viticulture in recent years through sudden changes in temperature and heavy rainfall or drought.
Growing grapes is a long-term commitment that requires at least five years before the newly planted vines give high-quality grapes. Changes in weather conditions (an average of 30 years), such as variations in temperature and humidity, late spring frosts, and early autumn frosts, floods, and drought, lead to certain diseases that affect grapes (Iliescu et al., 2019, Irimia et al., 2018).The health benefits of GP polyphenols have been the great interest of researchers, food industry(Beres et al., 2017), cosmetics (Beres et al., 2017; Maluf et al., 2018) and animal nutrition (Beres et al., 2017; Chedea et al., 2019; Chedea et al., 2018). In addition to phenolic antioxidants (Cotea et al., 2018), GPs also contain significant amount of lipids, proteins, nondigestiblefibre and minerals (Yu and Ahmedna, 2012). Due to these interests in GP valorisation this work presents the GP yields for some winegrapescultivars and clones cultivated in Târnave vineyards in the changing climatic conditions of the year 2020.

MATERIALS AND METHODS

Twenty-five winegrape cultivars: 11 cultivars including 8 white cultivars and 3 red cultivars; 13 clones for white wine, homologated at SCDVV Blaj as well as 1 red hybrid were analysed in this study.
Table 1 presents all the cultivars as well as their harvesting time, vineyard and pressing method.After harvesting the wine grapes were transported to the winemaking unit, were weighted and the berries were taken from the clusters by destemming. In function of the grapes mass's taken for vinification (Table 2) the destemming was done as following: for the mass less than 100 kg (e.g. Muscat-Ottonel 12 Bl from Crăciunelu de Jos, Syrah, Amurg) the manual crusher/destemmer Marchisio Baby Mano Tram (Italy) was used, for the mass between 101kg-1000 kg (e.g. Fetească Neagră, Traminer roz-60 Bl, Roze Blaj), the electrical crusher/destemmerGrifo DMC (Italy) was used, and for masses higher than 1001 kg (Feteasca Regală-21 Bl, Pinot-gris-34 Bl, Riesling Italian-3 Bl), the mechanical-horizontal destemmer /crusher was used. After destemming the resulted clusters were weighted and their percentage from the total grape mass was calculated. The grapes' pressing was done either by small pneumatic (for 90 kg), either by big pneumatic presser (for 1100 kg) as indicates Table 1.
For the resulting must different parameters were measured: the volume and weight of free run must, the free run must's content in sugar and its density, the volume and weight of the press must, the press must content in sugar, and its density. Sugar content in the must was measured using a refractometerKubler TA 25 no 656/91 (Germany).
In this measurement the unit vol. alc. was used. The density of the must was measured using a densitometer M.D.-A. (Romania) (Voşloban et al., 2020).The mass of total sugar free-run or pressed must (Kg) resulted from multiplication the free-run or pressed must volume with its sugar concentration (free run or pressed must sugar concentration in g/L) (Voşloban et al., 2020).Total sugar (of free-run or pressed must in kg) = Volume of the must x Sugar concentration.
The resulted GP was weighted and its yield was calculated. The percentage of losses was also calculated (Voşloban et al., 2020). Finally the yields for POD wine and table wine was calculated as following: Yield=(Amount of wine or GP/ Amount of grapes)x100.

Table 1. Studied wine grapecultivars, their harvesting time, vineyard location and pressing method

Cultivar/Clone/Hybrid	Harvesting data	Location	Pressing method
Cultivars			
White cultivars			
Blasius	01.10.2020	Crăciunelu de Jos	small pneumatic presser
Ezerfurt	29.10.2020	Crăciunelu de Jos	small pneumatic presser
Furmint	29.10.2020	Crăciunelu de Jos	small pneumatic presser
Hibernal	07.10.2020	Crăciunelu de Jos	small pneumatic presser
Radames	30.09.2020	Crăciunelu de Jos	small pneumatic presser
RozeBlaj	01.10.2020	Crăciunelu de Jos	small pneumatic presser
Rubin	25.09.2020	Crăciunelu de Jos	small pneumatic presser
Selena	25.09.2020	Crăciunelu de Jos	small pneumatic presser
Red cultivars			
Amurg	09.10.2020	Crăciunelu de Jos	small pneumatic presser
Fetească Neagră	24.09.2020	Crăciunelu de Jos	small pneumatic presser
Syrah	09.10.2020	Crăciunelu de Jos	small pneumatic presser
Clones (white cultivars)			
Fetească Albă-29 Bl	17-19.10.2020	Crăciunelu de Jos	big pneumatic presser
Fetească Regală-21 Bl	30.09-02.10.2020	Crăciunelu de Jos	big pneumatic presser
Ioardană 9-1 Bl	01.10.2020	Crăciunelu de Jos	small pneumatic presser
Muscat Ottonel-12 Bl	15-16.09.2020	Ciumbrud	big pneumatic presser
Muscat Ottonel-12 Bl	05.10.2020	Crăciunelu de Jos	small pneumatic presser
Neuburger-10 Bl	01.10.2020	Crăciunelu de Jos	small pneumatic presser
Pinot gris 11 Bl	05.10.2020	Blaj	small pneumatic presser
Pinot gris 18-5	05.10.2020	Blaj	small pneumatic presser
Pinot gris -34Bl	16.10.2020	Crăciunelu de Jos	big pneumatic presser
Riesling italian-3 Bl	21-22.09.2020	Ciumbrud	big pneumatic presser
Riesling italian-3 Bl	14-19.10.2020	Crăciunelu de Jos	big pneumatic presser
Riesling italian 18-15	29.10.2020	Blaj	small pneumatic presser
Riesling de Rhin 7-2 Bl	30.09.2020	Crăciunelu de Jos	small pneumatic presser
Sauvignon blanc-9 Bl	07-15.10.2020	Crăciunelu de Jos	big pneumatic presser
Traminer roz-60 Bl	16-17.10.2020	Craciunelu de Jos	big pneumatic presser
Hybrids			
Red cultivar			
Regent	10.09.2020	Blaj	small pneumatic presser

The experimental data was analyzed with the program Statview 5.0 performing one-way analysis of variance (ANOVA), followed by a Fisher protected least significant difference (PSLD) test. P values lower than 0.05 were considered significant while p values between 0.05 and 0.1 were considered as tendencies.

RESULTS AND DISCUSSIONS

Twenty-five winegrape cultivars: 11cultivars including 8 white cultivars and 3 red cultivars; 13 clones for white wine, homologated at SCDVV Blaj as well as 1 red hybrid were analysed in this study. Most cultivars cultivated in Transylvania, are white cultivars, because these vineyards belong to the B area, according to the UE zoning (Soare et al., 2010.). Following the technological line of grapes vinification, Table 2 shows the yield of clusters, wich is calculated after crushing-desteamming the grapes.

The desteamming is done by the removal of clusters in order to have a good quality wine and also GP. In our case the percentage of clusters is between 6.1 and 6.7 as Table 2 indicates. The results are in accordance to those presented in the work of Voşloban et al. (2020) also. Ţârdea et al. (2010), indicate that the clusters represent 3-8% of the grape mass and that their chemical composition is close to the one of leaves and tendrils (Ţârdea et al. 2010).

The winemakers choose to make the desteamming because during the vinification process in the clusters take place some osmotic processes as it would be the passage of the clusters' water in mustwhich is hipertonic rich in sugar, and also because during the alcoholic fermentation a part of the formed alcohol is absorbed by the clusters (Ţârdea et al. 2010, Voşloban et al. 2020).

During the crushing-desteamming process the free-run must is collected from which after fermentation the high quality POD wine is obtained. The crushed and desteammed matrix after collecting the free-run must is further pressed- in our case either with a small pneumatic presser, either with a big pneumatic presser in function of the pressed quantity (Table 1)- and the pressing must results. The pressing must is further the base of a lower quality wine like table wine.

For the resulting free-run and pressing must different parameters were measured and calculated (Table 2): volume, mass, sugar concentration, total sugar content and density. In terms of sugar concentration of the free run and pressing must, the limits were between 201.27 g/L for Fetească Regală- 21 Bl, 202.5 g/L for Riesling italian-3Bl, 204.85 g/L for Sauvignon blanc-9Bl and 236 g/L for Pinot gris-34 Bl.The results show a wine yield between 54% and 68 %, with the highest value for the cultivars Selena and Furmint, the clones Pinot gris-34 Bl and Fetească Regală-21 Bl (Table 2). The lowest yield (54%) was determined for the cultivar Hibernal and the clone Pinot gris 18-5 had also a low yield of 56%. After pressing the matrix resulted after crushing-destemming process, the grape pomace was collected and it was registered a GP yield between 34.09% for Pinot gris 18-5, 33.43% for Hibernal cultivar and 18.09% for Pinot gris 34 Bl, 18.56% for Furmint cultivar.

GP's chemical composition and its generation yield, can vary depending on factors such as environmental conditions, place of origin, grape cultivar, harvest period and various vinification techniques (Bettio, 2008). In this context the climatic conditions influence the production of GP, as they influence the growth and development of the grapes. For instance from our results we can see that the two cultivars of Pinot gris, Pinot gris 18-5 and Pinot gris 34 Bl are situated at extremes concerning the amount of GP generation. The differences might be explained by the fact that they were cultivated in different plantations, Pinot gris 18-5 at Blaj and Pinot gris 34 Bl at Crăciunelu de Jos (Table 1) so we can have a terroir

influence and that the grapes havesting dates are at a difference of ten days, 05.10.2020 for Pinot gris 18-5 and 16.10.2020 for Pinot gris 34 Bl respectively.

Târnave vineyard classically produces white dry POD wines. In order to check if there is any significant difference between the white cultivars and white clones homologated at SCDVV Blaj and cultivated in Târnave vineyard, in terms of GP production, we performed a statistical analysis.Figure 1 shows that there is no significant difference between these two groups (p=0.5474).

Table 2. Technological caracteristics of the studied grape cultivars, clones and hybrids at vinification

Cultivar/ Clone/ Hybrid	Vinified grapes		Clusters	Resulting grape must									Wine yield %			Grape pomace yield		Losses	
				Free run must					Pressing must										
				Volume	Sugar Concentration	Density	Mass (Kg)	Total sugar (Kg)	Volume (L)	Sugar Concentration	Density	Mass (Kg)	Total sugar (Kg)	POD	Table wine	POD+ Table wine	(Kg)	(%)	(Kg)	(%)
	(Kg)	(Kg)	(%)	(L)	(g/L)					(g/L)										
Cultivars																			
White																			
Blasius	158	10	6,2	79	210	1,089	86	17	20	210	1,089	22	4	50	13	63	38	24,05	2	1,4
Ezerfurt	780	49	6,3	390	210	1,089	425	82	117	210	1,089	127	25	50	15	65	170	21,79	9	1
Furmint	1390	89	6,4	737	209	1,088	802	154	209	207	1,0881	227	43	53	15	68	258	18,56	14	1
Hibernal	332	21	6,4	136	205	1,087	148	28	43	205	1,0872	47	9	47	13	54	111	33,43	5	1,5
Radames	307	19	6,3	153	208	1,088	166	32	49	208	1,0881	53	10	50	16	66	65	21,17	4	1,3
Roze Blaj	239	15	6,3	120	207	1,088	131	25	33	207	1,0881	36	7	50	14	64	54	22,59	3	1,2
Rubin	212	13	6,3	108	209	1,089	118	23	34	209	1,0885	37	7	51	16	67	41	19,33	3	1,3
Selena	213	13	6,3	117	210	1,089	127	25	28	210	1,089	30	6	55	13	68	40	18,77	3	1,2
Red																			
Amurg	95	6	6,5	47	207	1,088	51	10	12	207	1,0881	13	2	50	13	63	24	25,26	1	1,2
Fetească Neagră	231	14	6,2	120	208	1,088	131	25	35	208	1,0881	38	7	52	15	67	45	19,48	3	1,4
Syrah	95	6	6,4	48	208	1,088	52	10	15	208	1,0881	16	3	51	16	67	20	21,05	1	1,1
Clones (white)																			
Fetească alba 29 Bl	6390	407	6,33	3211	206,7	1,088	3489	653	1069	206,7	1,0877	1138	215	51	15	66	1266	20,58	79	1,3
Fetească regală- 21 Bl	22870	1409	6,1	12752	201,3	1,086	13852	2591	2885	201,3	1,0856	3133	585	56	12	68	4248	18,98	228	1,1
Iordană 9-1 Bl	110	7	6,4	55	205	1,087	60	11	16	205	1,0872	17	3	50	15	65	25	22,72	1	1,3
Muscat Ottonel-12 Bl *Ciumbrud*	7800	523	6,7	4290	209	1,089	4670	896	780	209	1,0885	849	163	55	10	65	1683	21	75	1
Muscat Ottonel-12 Bl *Crăciunelu*	80	5	6,5	40	210	1,089	43	8	10	210	1,089	11	2	50	12	62	20	25	1	1,4
Neuburger- 10 Bl	81	5	6,1	40	209	1,089	44	8	12	209	1,0885	13	2	50	15	65	18	22,22	1	1,2
Pinot Gris 34 Bl	2150	133	6,7	1183	236	1,1	1300	279	279	236	1,0996	307	279	55	13	68	389	18,09	21	1
Pinot gris 11 Bl	69	4	6,2	35	210	1,089	38	7	7	210	1,089	8	1	50	10	60	18	26,08	1	1,4
Pinot gris 18-5	88	5	6,1	38	210	1,089	41	8	10	210	1,089	11	2	43	13	56	30	34,09	1	1,5
Riesling Italian 18-15	150	9	6,3	75	210	1,089	82	16	19	210	1,089	21	4	50	13	63	36	24	2	1,1
Riesling Italian-3 Bl	34140	2151	6,3	9708	202,5	1,086	19220	3685	3982	202,5	1,0859	4297	785	55	13	67	7069	20,31	351	1,1
Riesling de Rhin 7-2 Bl	180	12	6,4	90	210	1,089	98	19	29	210	1,089	32	6	50	16	66	36	20	2	1,1
Sauvignon blanc-9 Bl	44880	2785	6,22	23287	204,9	1,087	25311	4760	6749	204,9	1,0871	7335	1380	51	16	67	9000	19,83	449	1
Traminer Roz-60 Bl	7700	485	6,3	4235	218	1,092	4265	923	847	218	1,0922	925	185	55	11	66	1565	20,32	100	1,3
Hybrids																			
Red																			
Regent	70	5	6,5	35	210	1,089	38	7	10	210	1,089	11	2	50	15	65	15	21,42	1	1,2

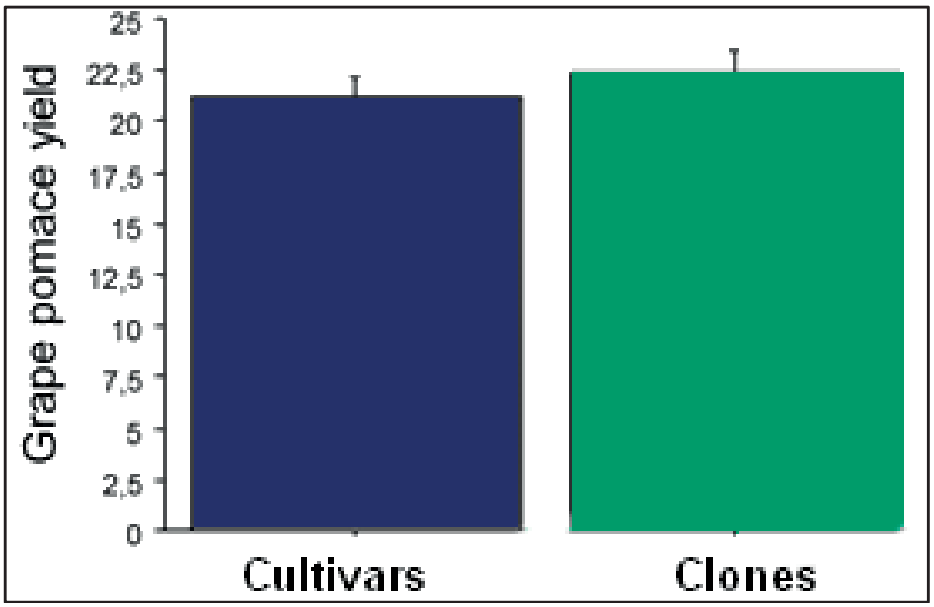

Figure 1. Grape pomace yields for the white cultivars and clones homologated a SCDVV Blaj and cultivated in Târnave vineyard

In the year 2020, the most critical period, climatically, with a negative effect on the plant, was the period of heavy rains in June that delayed the onset of flowering on the vine, and where the plant bloomed there were losses in fruit set.Also, the delay of the flowering period, by about 10 days, created a disturbance of the grapes' veraison. The veraison of grapes was particularly atypical and uneven.The cooling of the nights, in September, had a negative influence on the process of technological maturation of the grapes, with an influence on the sugar/ acidity balance in the must.The values for the active and useful thermal balance, during the vegetation period 2020 are slightly higher than the multiannual value, due to the higher temperatures in March and April. The global thermal balance has lower values until June and exceeds the multiannual average starting with July.

CONCLUSIONS

In the climatic conditions of the year 2020 with heavy rains in June and cold nights in September, the highest amounts of GP are obtained from the white cultivarHibernal (GP yield33.43% and sugar concentration in must 205 g/L) and from the white cultivar Pinot gris 18-5(GP yield34.09% and sugar concentration in must 210 g/L). Our data show that the harvesting time and the terroir influence the GP yield.

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EVALUATION OF THE AGROBIOLOGICAL AND TECHNOLOGICAL POTENTIAL OF SOME VALUABLE HYBRID ELITE OBTAINED AT R.D.S.V.O. ODOBEŞTI

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ABSTRACT

Over time at Research and Development Stationfor Viticulture and Oenology Odobeşti, numerous intra- and interspecific sexual hybridizations have been carried out, in order to obtain new cultivars, with high productive and qualitative potential, with disease tolerances, resistant to stress factors, very well adapted to the climatic conditions specific to Vrancea vineyards.Thus, valuable hybrid elites were obtained, which represent a permanent source for the selection, approval and promotion of new grape cultivars. In order to evaluate the agrobiological and technological potential of some valuable hybrid elites, in the period 2016 - 2018, two elites were studied: 'E.H. 10-1-6' and 'E.H. 6-1-1'. The results obtained from this study showed that these hybrid elites have a high productive and qualitative potential, show high biological resistance to the main diseases of the vine, and can be proposed for approval in order to improve the national assortment, in the context of sustainable viticulture.

Keywords: vines, breeding, resistance, cultivar

INTRODUCTION

The productivity, quality and adaptability of vine cultivars are very complex characteristics that depend on the genetic traits (inherited genotype or genetic dowry) of each cultivar, environmental conditions and the interaction between genotype and environment (Sestraș, 2004). Research conducted in recent decades in our country, has led to the production of valuable vine genotypes, with high tolerance to disease, drought and frost resistant.The scientific activity of vine improvement was and remains a strategic objective of tradition and continuity at Research and Development Stationfor Viticulture and Oenology Odobeşti (Bosoi et al., 2017, 2018; Puşcalău et al., 2018). In this context, in the last six years, four grape cultivars have been approved, with different production directions: a cultivar for table grapes 'Putna'(2014), a cultivar for superior white wines 'Vrancea'(2018), a cultivar for tinctorial red wines 'Măgura'(2014) and a cultivar for red and rose wines with genetic resistance 'Remus' (2016).

MATERIALS AND METHODS

The research was performed at the Research and Development Stationfor Viticulture and Oenology (RDSVO) Odobeşti, between 2016 and 2018. The biological material was represented by two valuable hybrid elites: the hybrid elite 10-1-6 obtained by crossing the

'Traminer roz' cultivar with the 'Isabella' interspecific hybrid and hybrid elite 6-1-1 obtained by crossing the hybrid combination 'Traminer x Armaş' with the 'Şarba' cultivar. The 'Fetească Regală' cultivar cultivated on large areas in the Odobeşti, Coteşti and Panciu vineyards, was studied as a witness.

The hybrid elites were characterized ampelographic, the phenological spectrum was monitored, were made observation and determinations regarding the elements of fertility and productivity, behavior at the main diseases of the vine, established by assessing with grades from 1 to 9 according to the resistance scale developed by the O.I.V. (2009), quantitative and qualitative potential of grape production.

RESULTS AND DISCUSSIONS

Ampelograpfic characterizationof the hybrid elite 10-1-6.At disbudding, the rosette is light green, slightly fluffy. The shoot is glabrous and has slightly intense anthocyanin coloration on the sunny side. The adult leaf is medium in size, pentalobate, slightly embossed, glabrous on the upper face and fluffy on the lower face, with the upper lateral sinuses open and the petiolar sinus open in the shape of a lyre. The flower is a normal hermaphrodite, type 5.

The grapes have a conical shape, rarely cylindrical, are medium to large in size and have medium compactness. The berries are medium-sized, spherical in shape, with pink skin, darker on the sunny side. The pulp does not have anthocyanin coloration, it is juicy, slightly firm, without specific taste (Fig. 1 and Fig. 2).

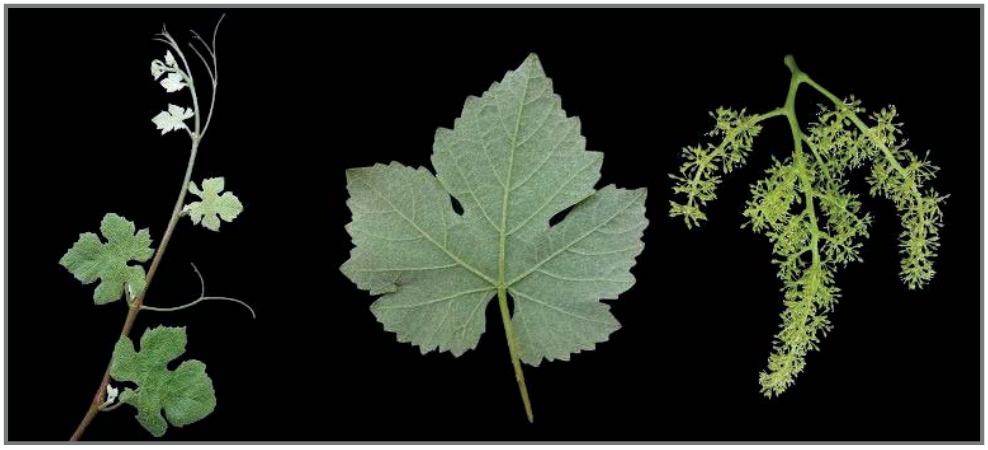

Figure 1. Elite hybrid 10-1-6 (shoot, leaf - lower side, inflorescence)

Figure 2. Elite hybrid 10-1-6 (shoot tip, leaf – upper side, grape)

Ampelograpfic characterization of the hybrid elite 6-1-1.At disbudding, the rosette is light green, slightly fluffy. The shoot is glabrous, with entirely green internodes with longitudinal striations. The adult leaf is small, pentalobate, slightly embossed, glabrous on the upper face. The superior lateral sinuses are deep, with the lobes slightly open, the lower ones open, and the petiolar sinus is semi-open. The flower is hermaphroditic, type 5.
The grapes are small, have a cylindrical shape, very rarely conical, with dense berries. The berries are small, spherical in shape, with golden-yellow skin, with more intense golden hues on the sunny side.The pulp does not have anthocyanin coloration, it is juicy, with a balanced taste, slightly aromatic (Fig. 3 and Fig. 4).

Figure 3. Elite hybrid 6-1-1 (shoot, leaf - lower side, inflorescence)

Figure 4. Elite hybrid 6-1-1 (shoot tip, leaf – upper side, grape)

Climatic conditions. The research period (years 2016 - 2018) was characterized by high heliothermal availability, the thermal regime expressed by the average annual temperatures (ºC) and the sum of the useful temperature degrees (∑ºtu) registering values much higher than the multiannual ones (Table 1). Compared to the multiannual values (10.5 °C, respectively 1604.1 °C), the average annual temperature varied between 12.0 °C and 13.2 °C, and the sum of the useful temperature degrees (∑ºtu), between 1933 , 6 °C and 2072.8 °C. The rainfall regime was a surplus during the vegetation period in all three years of study (between 613.2 mm and 910.6 mm), compared to the multiannual value (431.2 mm).

Table 1. The main climatic parameters from the study period (Odobeşti, 2016-2018)*

Climate parameter / Month	Average temperature (°C)				The sum of the useful temperature degrees (°C)				Rainfall (mm)			
	Multi annual	2015 / 2016	2016 / 2017	2017 / 2018	Multi annual	2015 / 2016	2016 / 2017	2017 / 2018	Multi annual	2015 / 2016	2016 / 2017	2017 / 2018
XI	5.1	9.2	5.3	6.5	9.5	45.7	5.7	1.4	45.8	108.0	68.2	70.2
XII	0.2	4.2	2.1	3.7	0.4	1.9	3.5	0.2	41.6	4.2	0.6	37.2
I	-1.6	-0.9	-2.8	0.2	0.1	0.0	0.0	0.0	34.3	6.4	10.2	16.6
II	-0.1	6.4	1.0	0.3	0.7	12.7	3.1	0.0	32.4	11.6	30.8	52.8
III	4.4	8.2	9.5	2.9	12.2	18.4	28.8	3.8	32.0	52.8	24.4	29.0
IV	10.9	14.2	10.9	16.3	66.9	144.2	61.3	189.2	48.4	131.2	71.8	1.8
V	16.4	16.7	18.2	20.4	212.8	206.3	250.3	323.6	74.3	151.8	31.6	20.4
VI	20.0	22.7	22.4	22.5	308.9	382.6	403.0	375.5	84.1	138.4	111.4	138.6
VII	22.0	24.6	22.6	22.1	371.7	452.3	391.5	374.0	78.2	13.0	93.4	181.8
VIII	21.2	23.3	23.9	23.9	351.1	411.2	431.5	429.4	59.1	188.8	47.6	42.2
IX	16.7	19.6	18.9	18.2	205.4	281.4	267.7	247.8	45.0	71.4	51.8	21.0
X	10.8	9.6	12.3	14.1	64.4	34.5	87.2	127.9	42.1	216.0	84.8	1.6
Annual average/ amount	10.5	13.2	12.0	12.6	1604.1	1991.2	33.6	2072.8	617.3	1093.6	626.6	613.2
during the vegetation period	16.9	18.7	18.5	19.6	1581.2	1912.5	1892.5	2067.4	431.2	910.6	492.4	407.4

*Data provided by the weather station AgroExpert of R.D.S.V.O. Odobeşti

The phenological spectrum. The phenology of the hybrid elites studied is presented in Table 2. In the climatic conditions specific to the study period (2016 - 2018) the growth and development processes of the vineevolved normally.

Table 2. The dynamic of the main phenological stages (Odobeşti, 2016 -2018)

The hybrid elite	Year	The phenological stages								Active vegetation period (days)
		Disbudding		Flowering		Veraison		Full ripening		
		Date	∑tu (°C)	Date	∑tu (°C)	Date	∑tu (°C)	Date	∑tu (°C)	
E.H. 10-1-6	2016	06.IV	56.0	30. V	369.8	06.VIII	1309.1	25.IX	1879.6	166
	2017	15.IV	65.8	03.VI	367.9	10 VIII	1306.4	18.IX	1777.4	157
	2018	17.IV	84.9	23.V	410.0	03.VIII	1307.2	10.IX	1807.9	147
Average		**13.IV**	**68.9**	**29.V**	**382,6**	**06.VIII**	**1307.6**	**18.IX**	**1821.6**	**157**
E.H. 6-1-1	2016	06.IV	56.0	30.V	369.8	01.VIII	1234.4	03.IX	1662.2	151
	2017	13.IV	57.9	01.VI	360.6	03.VIII	1193.1	08.IX	1655.1	148
	2018	15.IV	71.8	21 V	389.2	21.VII	1143.7	01.IX	1709.7	140
Average		**11.IV**	**61.9**	**27.V**	**373.2**	**28.VII**	**1190.4**	**04.IX**	**1675.7**	**146**
Fetească Regală (Control)	2016	09.IV	76.2	30.V	369.8	04.VIII	1277.3	06.IX	1699.9	151
	2017	14.IV	61.3	01.VI	360.6	03.VIII	1193.1	14.IX	1725.7	154
	2018	16.IV	75.3	20.V	379.5	22.VII	1155.6	04.IX	1752.6	142
Average		**13.IV**	**70.9**	**27.V**	**370.0**	**30.VIII**	**1208.7**	**08.IX**	**1726.1**	**149**

The full ripening of the grapes took place at the hybrid elite 10-1-6 between September 18 and 25, with four to 10 days later compared to the control cultivar, and in the first decade of the September at the hybrid elite 6-1-1, about a week earlier than the 'Fetească Regală' cultivar (Control).

Fertility and productivity characteristics. The fertility and productivity characteristics of the hybrid elites studied, assessed by the percentage of fertile shoots, fertility coefficients

(absolute and relative) and productivity indices (absolute and relative) show lower values compared to the control. In contrast, due to the higher average weight of the grapes, the hybrid elite 10-1-6 showed values of productivity indices higher than the control cultivar (Table 3).

Table 3. The fertility and productivity characteristics (Odobeşti, average data 2016-2018)

The hybrid elite	Fertile shoots (%)	Fertility coefficients		Average weight grapes (g)	Productivity indices	
		Relative	Absolute		Relative	Absolute
E.H. 6-1-1	64	0,68	1,08	134	95	151
E.H. 10-1-6	62	0,78	1,20	267	208	320
Fetească Regală (Control)	75	1,02	1,31	138	141	181

Behavior at the main diseases of the vine. The biological resistance to the main cryptogamic diseases of the vine appreciated according to the descriptors OIV 452, 453, 455 456, 458 şi 459 is presented in the table 4.

Table 4. The behavior at the main diseases of the vine

The hybrid elite	Downy mildew (*Plasmopara viticola*)		Powdery mildew *(Uncinula necator)*		Gray rot *(Botrytis cinerea)*	
	Leaf OIV 452	Grape OIV 453	Leaf OIV 455	Grape OIV 456	Leaf OIV 458	Grape OIV 459
E.H. 6-1-1	7	7-9	7	7-9	7-9	5
E.H. 10-1-6	7	9	7	7-9	7-9	5-7
Fetească Regală (Control)	7	7	7	7	7	5

The obtained data show that during the study period (2016-2018), years that registered during the vegetation period favorable climatic conditions for the attack of cryptogamic diseases, under the application of the scheme of anticryptogamic treatments, the hybrid elites studied showed high resistance, superior to the control cultivar - Fetească Regală. The elite hybrid6/1/10 stood out by high biological resistance to the three main diseases of the vine.

Technological characteristics of grape production. The study of the technological characteristics of grape production complemented the knowledge elements for the hybrid elites studied (Table 5). The number of grapes per vine and the obtained production confirm the fertility data for the two hybrid elites compared to the control. The hybrid elite 10-1-6 was noted with a production of 5.87 kg/vine, superior to the control cultivar (4.14 kg/vine).

Table 5. The quantitative and qualitative characteristics of grapes and juice (average data, Odobeşti, 2016-2018)

The hybrid elite	No. bunch/ vine	Weight grapes (g)	Weight 100 berry (g)	Production grape		The juice	
				kg/vine	kg/ha	Sugars g/l	Total acidity g/l H_2SO_4
E.H. 6-1-1	24	135	136	3.22	12.197	236	3.12
E.H. 10-1-6	22	267	202	5.87	22.235	217	4.74
Fetească Regală (Control)	30	138	139	4.14	15.682	191	4.16

From a qualitative point of view, the hybrid elites studied were above the control cultivar, with higher values of sugar content in must (236 g/l for 'E.H. 10-1-6' and 217 g/l for 'EH 6-1-

1', respectively 191 g/l for the 'Fetească Regală' cultivar). A good value of the total acidity relative to the sugar content was achieved by the hybrid elite 10-1-6 (4.74 g/l H_2SO_4). Lower total acidity was recorded by the hybrid elite 6-1-1 (3.12 g/l H_2SO_4), under conditions of high sugar accumulation.

CONCLUSIONS

- The two hybrid elites studied at R.D.S.V.O. Odobeşti during the years 2016 - 2018, presented valuable agrobiological and agro-productive characteristics which recommends their proposal for homologation and promotion in culture for the diversification of the local assortment of cultivars with high ecological plasticity.
- The hybrid elite 10-1-6 was distinguished by a high productive and qualitative potential, as well as a good genetic resistance to the main diseases of the vine (downy mildew, powdery mildew and gray rot).
- The hybrid elite 6-1-1 stood out with good results regarding the qualitative and productive potential, as well as by a high tolerance to the main cryptogamic diseases.

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ANTHOCYANINS PROFILES OF SOME AUTOCHTONOUS GRAPEVINE CULTIVARS FOR RED WINES, CULTIVATED AT DRĂGĂȘANI, ROMÂNIA

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ABSTRACT

The Dragasani vineyard is one of the most ancient existing in Romania being famous for obtaining red and white wines of the highest quality. The present study has performed during five years (2005-2009), focusing on the anthocyanin profiles of the autochthonous Novac grapevine cultivar, in comparison with Cabernet Sauvignon cultivar which, also provides wines of high quality. The obtained results put into evidence, a special potential and chromatic structure in case of the grapes and wines characteristics for these autochthonous cultivars, which are very closed to those shown by the Cabernet Sauvignon. For all the cultivars studied, the amounts of cationical flavidium within the anthocyanin matter do keep continuously was growing, while acidity decreased and glucides was accumulated. However, at some moment, these synthesis indices of the cromatical structure's quality begin to decline. Depending of the viticultural year it is the genetical nature of the respective anthocyanin pigments to differentiate through the involvements of each of the 3 categories of pigments (yellow, red or blue). During the wine's evolution (maturation and aging), due to the *modification of its cromatical structure, the ratio between the yellow and red* pigments does confer to the wine a colour nuance which continues to increase, significantly improving the wine's visual attraction. For the autochthonous grapevine cultivars for red wines, their antocyanins potential their respective qualities do enable us to state that on the basis of the quality of the raw materials, wine types of high qualities could be created, being so endowed that they could bear as well an origin denomination.

Keywords*:* wine, cromatic structure, pigments, anthocyanins

INTRODUCTION

The vineyard of Dragasani is one of the most ancient in Romania. It is a former Dacian vineyard, famous for its vocation which allowed to obtain wines of the highest quality, as well as others aromatic or white ones. The Dragasani vineyard, in the matter of its oenoclimate, is situated within the A3 zone. Its viticultural centers are located upon hills. They mostly produce red and aromatic wines while, as a subsidiary specialty, they also provide white wines. The vineyard benefit from climate opportunities that allow, the synthesis in the grapes of whatever among its necessary constituents, no matter how complex their chemical structure might be. However, the abundance of these opportunities is not accompanied by climate's toughness's, that could in any case simultaneously, cause deteriorations of their constitutive harmony, by wastes or degradations (Popa, A., 2007; 2012). The Dragasani red wines are defined through more stamina than others, by the richness of their colours, the beauty they have in terms of their hue by their great propension for aging. Their main virtues are related to the way in which these wines

achieve an accurate expression of the quality assets held by the types from which they comes (Macici, M., 2008). The delicate specific red wines are obtained from grapes cultivars such as: Cabernet Sauvignon, Pinot Noir, Fetească Neagră. More recently, at Drăgăşani Station for Viticulture and Oenology: Negru de Drăgăşani and Novac grapevine cultivars were created. These two newly created cultivars have as parents Negru Vartos (an ancient Romanian cultivar) and from Saperavi.
The studies performed concerning the amounts of anthocyanins that exist in grapes, musts and wines have pointed out the items that determine their accumulation in grapes, the structure in which they are aggregated , the role they must play in the hygienical and sanitary creation of status value of wines (Ribereau Gayon G., 1991, Fisher U., 1998, Vivas de Gaulejac N., et all., 2001, Otteneder H et all. 2001, Burus J et all., 2002, Saurey R et all. 2007, Teodorescu St., 1957, Teodorescu St. et all., 1987, Carbonneau A., 1993, Hamatschek J., 1991, Onescu Janina, 2008, Kontek Adriana, 1987, Ţârdea C et all., 2000; Cotea D.V. et all., 2009).

MATERIALS AND METHODS

The antocyanes from the berries of the grapes were determined through the Poissant Leon method. From the red wines anthocyanins were determined by spectrophotometrical method, through the pH difference. The difference between the optical densities of solutions with two different pH, read at 520 nm is directly proportional to the amount of anthocyanins contained by the wine. The concentration of anthocyanins was established following the standardizing graph curve, drawn upon the ground of collected data, and its expression is done in mg/L. The red wines'colour was determined in the visible domain through a spectrophotometry at the wavelengths of 420 nm (yellow component, 520 nm, for red component and at 620 nm for blue component, color intensity, hue, were calculated as follows:
Ic = DO 420 nm + DO 520 nm + DO 620 nm
Nc = DO 420 nm/DO 520 nm
dA = DO 520 nm – (DO 420 nm + DO 620 nm/2)/DO520 nm
The anthocyanins 'ratio combined with tannins (pvp%) and the polymerized pigments pp% was estimated. In order to follow the evolution of the grapes'ripening process, the methods recommended by the O.I.V. was applied.
The wines were obtained by the classical method of red winemaking. The wine's maturation was realized in oak tree wood vases (bariques) with a capacity of 220 litres, while the aging was made in bottles. Within the storage space, a temperature of about 10-12°C was ensured.

RESULTS AND DISCUSSIONS

In matters of quantity and quality, the colour of red depends not only on the anthocyanins content of the grapes which are the raw material, but also on the ratio of the primary winemaking, which is found by the oenological quantity called the technological reserve.

For the studied parameters, the obtained results, concerning the anthocyanins content, their extractability and the technological reserve are presented in Table 1 The data's ensemble analysis eloquently points out the fact that the Cabernet Sauvignon cultivar has the capacity to exploit the conditions offered by the Dragasani vineyard.

Table 1. Anthocyanins Contents, Extractability and Technological Reserve for anthocyanins for Some Grapevine Cultivars for Red Wines Cultivated in the Vineyard of Dragasani, Romania (Limits and Average Mean for 5 Years, 2005-2009)

Parameter	Moment of determining	Cultivar			
		Cabernet Sauvignon	Feteasca Neagra	Negru de Dragasani	Novac
Anthocyanins mg/kg of grapes	Full maturity	1354 -1495 1402	1061-1164 1099	1199-1270 1224	1384-1470 1398
	Phenolic anthocyanins maturity	1461-1570 1507	1208-1346 1276	1296-1460 1381	1422-1518 1485
	Technological maturity	1425-1540 1470	1203-1307 1255	1274-1415 1330	1487-1500 1494
Anthocyanins extractability %	Full maturity	48.9-52 50.2	48.6-50.2 49.7	47-49.5 48	45.9-49.1 47.3
	Phenolic anthocyanins maturity	53.1-56 54.4	50.6-54 52.5	52-53 52.5	48.6-53.1 52.1
	Technological maturity	54.8-57.3 56.1	53.4-56.7 54.9	52.6-56 54.5	52-56 55
Anthocyanins extractabile from grapes (technological reserve) mg	Full maturity	662.1-769 705	506.1-589 546	558-622 584	638-685 666
	Phenolic anthocyanins maturity	776-869 820	609-725 665	666-780 731	687-795 761
	Technological maturity	781-874 825	639-733 687	668-789 709	769-832 810

At its full maturity even in the viticultural years which, in climate matters, should present deficits, due to the kind's genetical nature, within the grapes' berries might be accumulated a concentrations in anthocyaninswhich would be enough to obtain red wines with a convenient colouring. In the viticultural years with a lot of heat and light, the anthocyanins amounts almost reach up to 1495 mg/kg of grapes, so it is a higher ratio. At the phenolic maturity, a phenol phase which occurs after full maturity, the grapes' contents in anthocyanins increases to high values.

At technological maturity, for all the the period the phenolic maturity was registered, but still they are excellent for obtaining red wines with valuable chromatic characteristics of high quality chosen.

For the Cabernet Sauvignon, the rations of extractible anthocyanins from the grapes' has been touched at maturation, as well as when it passed from full maturity to technological maturity. According to the content of anthocyanins's in grapes and the degree of their extraction, the level of the technological reserve also increase.

The data about the technological reserve show that richly colored red wines can also be obtained in situation where Cabernet Sauvignon grapes are harvested at their full maturity, a phenolic phase when the production reaches its stage of course if the other composition corresponds to the type of wine we would like to get it. If the Cabernet Sauvignon grapes have to be harvested at their phenolic and technological maturity, they would result in intensely coloured wines, the respective colour being comparable to that of similar wines issued from other viticultural areas which are famous for this production line.

For the Feteasca Neagra grapes, the anthocyanins potential is lower that of Cabernet Sauvignon.

The diference is dictated both by the genetical nature of respective cultivar but significantly by the high quantities of berries skins, since the Cabernet Sauvignon berries is smaller than that of Feteasca Neagra.

In the Feteasca Neagra grapes, the anthocyanins potential display chromatic characteristics which are influenced, as well as in the case of Cabernet Sauvignon, by the climate circumstances offered by the respective viticultural harvest year.

For all the three phenolic phases of the grape's maturation, the anthocyanins amounts have evolved increasingly, both from one year to another and, within a same year, they increased from full maturity to technological maturity.

For the extractible anthocyanins, the ratios increased from full maturity to technological one, even if for the grape berries of Feteasca Neagra, the anthocyanins sometimes decresed, between the phenolic maturity and the technological one.

For all maturities, either completely phenolic or technological, the registered technological reserve is able to ensure, for Feteasca Neagra wines, the required necessary colour to be included in superior categories, a place that is also guaranteed due to the other characteristics of the grapes, either in technology or in composition. Concerning the biological synthesis and accumulation capacities of the grapes regarding to the colouring matter from anthocyanins, at the Negru de Dragasani cultivar their they are not much smaller in the case of Cabernet Sauvignon cultivar.

The study of the anthocyanins content in grapes and that of their extractability recorded at the phenolic and technological maturity, allows us to conclude that the obtaining wines will have a good coloring intensity and beautiful hue.

In conditions of grape harvests that often exceed 9000 kg/ha, for the Novac cultivar the anthocyanins' content is quite closed to that of the Cabernet Sauvignon cultivar.

Compared to this latter for Novac grapes of the Novac kind, the degree of anthocyanins extractability is lower. For this reason, technological reserves are also smaller, but not with a considerable difference. For each of the stages of maturation of the Novac grapes, technological reserves are considered to be particularly favourable for obtaining of some intensity coloured wines, situated in this sense, near those of Cabernet Sauvignon and beyond those of Feteasca Neagra and Negru de Dragasani.

As a defining advantage for the quality of these types of wines, the red wines' colour is edified not only on the quality of anthocyanins matter in its constitution, but also by its chromatic structure, established by the the participation of the three categories of pigments (yellow, red and blue). Table 2 presents the results obtained.

At Cabernet Sauvignon, for the whole period of time, the yellow component was situated under 30%. The highest value of this component is usually recorded in viticultural years with an excess of precipitations but not sunny enough. The red component appears in higher proportions in the good viticultural years in matters of climate meaning warm and under a good light. The blue component seems to benefit from less favourable climate conditions. At the Feteasca Neagra grapes the chromatic structure of the anthocyanins colors matter as a whole, is characterized by a more accentued variability of the three components.

The yellow and the red components show higher values during viticultural years with lots of rains and less insolation. The complex anthocyanic chromatic structure from the grapes of the Feteasca Neagra cultivar, as a whole, proves that for the various climate conditions existing in several viticultural years, a corresponding variation is created, especially for the yellow and blue components, which impose conditions on the indices of the red component.

The first statement which can be presented on the quantized ensemble of the chromatic sizes which are specific to the chromatic structure of the anthocyanin extractions

from the grapes of the Negru de Dragasani refers to a certain persistence over time of the yellow component, less than 30%, for all the studied years that are warm and sunny, the blue pigments present higher rations in the rainy years, more chilly and with shorter isolation time during the phenolic phases of the grapes maturation. In grapes of the Novac cultivar, the chromatic structure of the anthocyanins matter was significantly similar to that of Negru de Dragasani grapes. At technological maturity, the sizes of the three components from the structures of the colouring matters within grapes are are found in the levels of the chromatic assets, represented by the colouring intensity of color (Ic), the hue (the colour's tonality – Nc) and by the flaviliumcations (dA%).

Table 2. Cromatic Structure of the Anthocyanins Complex from the Grapes of the Grapes Cultivars for Red Wines, Cultivated in the Vineyard Dragasani, Romania
(Limits and Average Mean for Five Years, 2005-2009) at Their Technological Maturity

Pigments	Specification	Cultivar			
		Cabernet Sauvignon	Feteasca Neagra	Negru de Dragasani	Novac
Yellow	D.O.420	0.807-0.915 0.850	0.69-0.809 0.769	0.796-0.886 0.828	0.873-0.898 0.887
	%	29.5-29.7 28.9	29.95-31.14 31.05	29.05-29.70 29.36	28.57-29.31 29
Red	D.O.520	1.649-1.919 1.809	1.406-1.579 1.519	1.614-1.850 1.725	1.807-1.920 1.867
	%	60.39-62.6 61.7	60.7-62 61.2	60.2-61.29 60.87	60.44-61.38 67
Blue	D.O.620	0.275-0.287 0.279	0.202-0.216 0.210	0.269-0.288 0.279	0.299-0.319 0.309
	%	9.19-10.1 9.62	7.94-9.20 8.53	9.56-10.20 9.85	9.66-10.40 10.10

Table 3 presents the solid reasons for which the grapes for red wines that are cultivated at Dragasani have a vocation for obtaining wines of high quality. The color intensity, the color's hue and the flaviliumcations of Cabernet Sauvignon wines place them in the category of great red wines. The Novac and Negru de Dragasani wines, in terms of their richness and color's beauty are close to those of Cabernet Sauvignon. All these obtained results lead us to the foresight the completion of the Dragasani red wines.

Table 3. Cromatic Characteristics of the Anthocyanins Extracts from the Grapes for Red Wines, Cultivated in the Vineyard of Dragasani Romania (Limits and Average Mean for Five Years, 2005-2009) at Technological Maturity

Chromatic characteristics	Cultivar			
	Cabernet Sauvignon	Feteasca Neagra	Negru de Dragasani	Novac
Color intensity (Ic)	2.735-3.140 2.940	2.305-2.589 2.570	2.760-3.20 2.83	2.99-3.135 3.080
Color tonality (Tc)	0.474-0.490 0.470	0.490-0.518 0.499	0.475-0.493 0.483	0.479-0.485 0.467
Flaviliumcations	67.25-70.66 69.80	66.51-67.89 67.35	66.92-70.65 69.66	67.26-68.7 68.2

The evolution of the various forms of the anthocyanins, the evolution in time of the elements of the chromatic structure, the evolution in time of the chromatic characteristics of the Cabernet Sauvignon wines are presented in Tables 4, 5 and 6.

An analysis in time of the chromatic elements in their evolution, starting with the separation of the must's fractions up to the age of 18 months underlines: the continuous decrease of the content of total anthocyanins, the continuous increase of the amount of anthocyanins combined with tannins, as well as the continuous increase of polymerized pigments, facts that are proven by the indices Antocyans combined with tannins (pvp), respectively Polymerized pigments (pp). If we had to compare the data for every stage of the evolution, we would notice the lower proportion for the combination of the anthocyanins for the aging process, in relation to the ratios from the maturation's stages.
The evolution of the pvp (%) index is another evidence attesting to these facts. The components of the anthocyanins complex also evolve, starting with the phases' separation up to the wine's age of 18 months: the yellow component is continuously increasing; the red component, taken into consideration for each of the stages, is on a decreasing trend: the blue component presents an evolution lacking constance, which is oscillating through ups and downs. The wine's chromatic assets evolve as÷ follows: the Ic = color intensity decreases continously; the Nc = the color tonality or nuance is continuously increasing: the flaviliumcations at first increase slightly, between separation of the phases and the wine's age of three mounts: further during the maturation and the aging processes, they progressively diminish from one stage to another.

Table 4. Evolutions of Various Forms of anthocyanins from the Cabernet Sauvignon Wine, Dragasani, Harvest of 2007

Oenological anthocyanins sizes	At the separation of the must's phases	Age of wines (months)					
		Maturation				Aging	
		3	6	9	12	15	18
Total amount of anthocyanins (mg/l)	808	778	759	722	676	602	591
Free anthocyanins (mg/l)	750.6	690	591.3	457.7	383.3	319.7	302.6
Free anthocyanins (%)	92.9	88.7	77.9	63.4	56.7	53.1	51.2
Combined anthocyanins (mg/l)	57.4	87.9	167.7	264.3	292.7	282.3	288.4
Combined anthocyanins (%)	7.1	11.3	22.1	26.6	43.3	46.9	48.8
index pvp %	11	20	27	31	34	36	37
index pp	8	32	34	38	42	44	50

index pvp = Antocyans combined with tannins
index pp = Polymerized pigments

Table 5. Evolution in Time of the Elements of Chromatic Structure for the Wine of Cabernet Sauvignon, Dragasani, Harvest 2007

Elements of chromatic structure of the anthocyanins complex	At the separation of the must's phases	Age of wines (months)					
		Maturation				Aging	
		3	6	9	12	15	18
D.O.420 nm	0.486	0.470	0.446	0.436	0.410	0.394	0.373
D.O. 520 nm	0.916	0.880	0.730	0.706	0.657	0.625	0.580
D.O.620 nm	0.210	0.199	0.180	0.177	0.170	0.166	0.152
Yellow pigments %	30.148	30.34	32.89	33.06	33.14	33.25	33.76
Red pigments%	56.823	56.81	53.83	53.52	53.11	52.74	52.49
Blue pigments%	13.027	12.85	13.27	13.42	13.74	14.50	13.75

At the Novac wines, the evolution of the anthocyanins forms, of the elements of the chromatic structure and of the chromatic assets are very similar to those of Cabernet

Sauvignon wines (Tables 7, 8 and 9), with the exception of rythms and durations, which are mostly different.

Table 6. Evolution in Time of the Chromatic Assets of the Cabernet Sauvignon Wine, Dragasani Harvest, 2007

Chromatic characteristics of the wine	At the separation of the must's phases	Age of wine (months)					
		Maturation				Aging	
		3	6	9	12	15	18
Color intensity (Ic)	1.612	1.549	1.356	1.319	1.237	1.185	1.105
Color tonality (Tc)	0.530	0.534	0.610	0.617	0.624	0.630	0.643
Flaviliumcations	61.91	62.03	57.09	56.64	55.86	55.20	54.82

Table 7. Evolutions of Various Forms of Antocyanes from the NovacWine, Dragasani, Harvest of 2007

Oenological anthocyanins sizes	At the separation of the must's phases	Age of wines (moths)					
		Maturation				Aging	
		3	6	9	12	15	18
Total amount of anthocyanins (mg/l)	821	784	769	730	667	651	635
Free anthocyanins (mg/l)	739.7	582.5	542.9	478.1	382.2	358.7	341
Free anthocyanins (%)	90.1	74.3	70.6	65.5	57.3	55.1	53.7
Combined anthocyanins (mg/l)	81.3	201.5	226.1	251.9	284.8	292.3	294
Combined anthocyanins (%)	9.9	25.7	29.4	34.5	42.7	44.9	46.3
Indice index pvp(%)	10	24	28	30	33	34	35
Indice index pp	9	30	32	36	38	40	41

Indice index pvp = Antocyans combined with tannins
Indice index pp = polymerized pigments

Table 8. Evolution in Time of the Chromatic Assets of the Novac Wine, Dragasani Harvest, 2007

Elements of chromatic structure of the anthocyanins complex	At the separation of the must's phases	Age of wines (months)					
		Maturation				Aging	
		3	6	9	12	15	18
D.O.420 nm	0.508	0.496	0.470	0.451	0.440	0.436	0.430
D.O. 520 nm	0.936	0.901	0.830	0.795	0.743	0.735	0.721
D.O.620 nm	0.231	0.227	0.204	0.190	0.183	0.180	0.173
Yellow pigments (%)	30.33	30.54	31.25	31.41	32.21	32.27	32.48
Red pigments (%)	55.88	55.48	55.19	55.36	54.39	54.40	54.46
Blue pigments (%)	13.79	13.98	13.56	13.23	13.40	13.32	13.06

Table 9. Evolution in Time of the Chromatic Assets of the NovacWine, Dragasani Harvest, 2007

Chromatic assets of the wine	At the separation of the must's phases	Age of wine (months)					
		Maturation				Aging	
		3	6	9	12	15	18
Colouring intensity (Ic)	1.675	1.624	1.504	1.436	1.366	1.351	1.324
Colour's tonality (Tc)	0.542	0.550	0.566	0.567	0.592	0.593	0.596
Flaviliumcations (dA%)	60.56	59.89	59.35	59.70	58.10	58.07	58.21

All these values of the chromatic sizes confirm the vocation for aging of the Novac wines.

CONCLUSIONS

- Studies carried out on the anthocyanins profile of some autochtonous grapes cultivars for red wines which are cultivated at Dragasani have pointed out, once again, the vocation of obtaining red wines of superior quality owned by this vineyard, as well as their genetic potential of which have the cultivars of Feteasca Neagra, Novac and Negru de Dragasani, allowing them to prosper into the paedo-climatic circumstances of Dragasani;
- The antocyanic profiles of the autochtonous cultivars Feteasca Neagra, Negru de Drasani and Novac in comparison with those of the Cabernet Sauvignon which at Dragasani provides wines of very high quality present some similar values;
- The participating of the three categories of pigments (yellow, red and blue) was proven to be a genetical character, which is dependent, in its size, upon the climates' conditions;
- During the wine's evolution (maturation and aging) the chromatic characteristics (the color's intensity and tonality) reach the valves that are the features of the great red wines;
- Amoung the sorts of grapes assortment for red wines existing at Dragasani, Feteasca Neagra, Negru de Dragasani and Novac cultivars should achieve an important weight.

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THE INFLUENCE OF CLIMATIC CONDITIONS ON OENOLOGICAL PARAMETERS OF SOME WINE CULTIVARS FROM DIFFERENT ROMANIAN VINEYARD

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ABSTRACT

A study concerning the influence of climatic conditions on the oenological parameters of wine cultivars was carried out using six wine cultivars under the climatic conditions of 2019. Wine samples were taken from Ştefăneşti–Argeş, Babadag and Drăgăşani viticultural centres. Determination of the oenological parameters has been made for the following wine cultivars: Riesling Italian, Fetească Regală, Sauvignon Blanc, Fetească Neagră, Merlot and Pinot Noir. The chemical parameters were determined using a Mindray BS 200 oenological analyser and the statistical analysis of the results was carried out using the Duncan test. A correlation was made between the parameters of the six wine cultivars from the three viticultural centres, focusing on the correlations between the total polyphenols of wines and their antioxidant activity.

Keywords: climate change, wine cultivar, chemical composition, polyphenols, antioxidant activity

INTRODUCTION

The global warming has caused many disturbances in the viticultural ecosystems, the vine cultivars being forced to modify their annual cycle of vegetation, with consequences, most often negative, for the quality and quantity of grape production andalsofor the resulting wines (Tănase, 2019). Already, negative effects of climate change in some vineyards on the globe, such as: earlier ripening of grapes, loss of acidity through respiration and a greater accumulation of sugar, were reported. Also, if the harvest takes place earlier than usual (August or September, instead of October in the northern hemisphere) and the vine is not irrigated, dehydrated grapes will result (Onache, 2018).

The quality of the wine is given by the physico-chemical composition of the wine and its organoleptic properties. In case of wine, that has a very complex composition, the quality remains a random feature, difficult to define and to establish (Geana, 2016).

Red wine is a tannic drink, because it contains polyphenols, which is not the case of other alcoholic beverages. Polyphenols are, in fact, very important constituents of wine and a greate interest is related to their antioxidant action. Polyphenols settle the taste and colour of wine. The most sustained beneficial action of polyphenols from wine is their antioxidant effect, which is achieved by the capture by polyphenols of free radicals circulated through the blood. (Woraratphoka, 2007; Valkom, 2007; Yang, 2009; Visnja, 2010; Bunea, 2012). Consequently, a vigorous research activity was initiated regarding the analysis of these

antioxidants in different grape products and on the preparation of extracts and wines with a high content of phenolic antioxidants (Heinonen, 1998).

MATERIALS AND METHODS

In this experiment, grape production and the resulted wines of 6 wine cultivars were studied in 3 winegrowing areas.on the specific climatic conditions of the year 2019. The wine cultivars studied were: Riesling Italian, Fetească Regală, Sauvignon blanc, Fetească Neagră, Merlot and Pinot Noir. Climate determinations have been made for each vineyard using meteo stations, as fallows: Golesca station (topographic data: 44°85'75"N and 24°95'77"E, 300m); Babadag station (Dobrogea) (topographic data: 44°94'05" N and 28°65'09"E, 250m) and Drăgăşani station (topographic data 44°69'56"N and 24°23'57"E, 195m). The climate data were collected between January and September 2019.
A very large set of chemical analyses were performed. For each wine was determined the following parameters: minimum alcohol content, total aciditыand anthocyanins content. The alcohol content, one of the most important parameters of wine quality, was determined by indirect method (hydrostatic balance method), after prior separation of alcohol from wine by distillation. Acidity provides physico-chemical stability of wine, gives colour, brightness and freshness of taste. To characterize the acidity of wine the following types of acidity were taken into consideration. The determination of total acidity was made by titration with bromothymo lblue(Giosanu, 2011).
The total content of polyphenolic compounds (CTCF) was determined by the Folin-Ciocalteu method. With the help of the oenological analyzer Mindray BS200 the organic acidswere determined. (Ţârdea, 2007).
The samples were analyzed according to the technique reported by Brand-Williams and others (1995), with some modifications. Briefly, 1 volume of sample (5 µL for red wine [1 : 1, v/v with methanol]) was added to 1 volume of 2.2-diphenyl-1picrylhydrazyl (DPPH•) (Sigma) 0.094 mM in methanol up to completing 1 mL. The free radical scavenging activity using the free radical DPPH• reaction was evaluated by measuring the absorbance at 515 nm after 60 min of reaction at 20°C using a spectrophotometer. The reaction was carried outin closed Eppendorf tubes shakenat 20°C and the results were expressed in gallic acid (Woraratphoka, 2007).
For the statistical interpretation of the results, the data were included in an Excel database and then statistically interpreted with the SPSS 14.0 program, which uses the Duncan test (multiple range test) for a 5% statistical assurance.All the biochemical determinations were carried out in three repetitions.

RESULTS AND DISCUSSIONS

The climatic values recorded in Ştefăneşti vineyard centre showed some peculiarities whose effects were found both in the course of the vegetative cycle and especially in the level and quality of the grape harvest. Among these specific features we can mention, as the most significant, the following:the low temperatures in the period of April – May, leading to a delay of the flowering by one week (3°C /April 1st, 5.9°C/ May 1st, 6.1°C /May 7th);the temperature in June didn't exceed 35°C, until 2 days (37°C June 25 and 35.6°C June 27)and in July there were not reported very high temperatures (maximum 37.8°C/July 21 and July 26);in two months (June and July)the temperatures during the night were quite low (14°C /June 03 - June 10 and 8.2°C /July 11) leding to the extension of the ripening period of the grapes (Table 1).

Table 1. Temperature dynamic duringApril-August 2019 in the three vineyard centres

Month	Air temperature			Rainfall (mm)	Huglin Index	Days without precipitation> 10 mm	Σ T global (°C)	Σ T active (°C)	Σ T useful (°C)
	T med (°C)	Average T min (°C)	Average T max (°C)						
Drăgăşani									
April	11.92	6.53	17.83	39.9	146.25	1	357.5	314.5	64.5
May	14.08	9.42	19.06	14.9	203.67	-	436.5	436.5	166.6
June	22.81	17	29.23	139.2	480.6	7	684.5	684.5	384.5
July	22.82	15.84	30.16	63.8	511.19	3	707.5	707.5	397.5
August	25.13	17.39	32.87	1	589	-	779	779	469
Babadag									
April	13.3	8.43	17.97	810.2	163.05	8	401	354.5	124.5
May	17.5	13.1	22.29	518	241.65	4	542.5	542.5	232.5
June	20.5	16.2	24.73	707.1	378.45	7	614.5	614.5	314.5
July	27.3	21.45	32.42	602.7	615.66	6	845	614.7	304.7
August	27.35	20.58	31.94	199.2	609	3	848	848	538
Ştefăneşti									
April	11.2	5.4	19.1	42.2	171	1	338.1	244.4	53.4
May	16.3	9.8	24.6	93.6	323.95	2	504.1	494.5	194.5
June	22.1	15.5	32.0	193.6	511.5	6	664.6	664.6	364.6
July	22.2	14.5	32.2	70.6	531.95	1	687.1	687.1	377.1
August	24.6	15.7	35.8	6.6	626.2	0	762.1	762.1	452.1

Huglin Index =[(Tmed – 10)+(Tmax - 10)]/2 x no.days of the month.
Σ T global = sum of average positive daily temperatures.
Σ T active = sum of average daily temperatures > 10°C.
Σ T useful = sum of differences between average daily temperature >10°C and the biological threshold for starting in the vegetation of the vine (10°C).

The climatic data in Drăgăşani vineyard didn't show any peculiarities in terms of temperatures, which were kept within the normal limits, specific for the Drăgăşani area. In March small peculiarities represented by a minimum temperature of -2°C (in March 15) and a maximum of 25°C (in March 18) and poor rainfall (only in March 18 were recorded 18.7 mm) were noticed. April was characterised by a minimum temperature of 2°C (April 3 and 21), a maximum of 25°C (April 27) and poor precipitation (with 15.4 mm rain in April 18). In May, climate data remained normal (22-28°C) for this rain-free area, only on May 31 was recorded a maximum temperature of 20°C. The ripening period of grapes (June-August) was deficient in terms of precipitation. Concerning the temperatures, only in August there were higher temperatures (36°C /August 10 and 11).Climate data from the Dobrogea vineyard showed some peculiarities, such as the low temperature in March (T max 5°C /March 25) and high rainfall (99.5mm /March 16 and 17; 99.8 mm / March 21and 22). April was evidenced by low maximum temperatures (7-18°C) and heavy rainfall (99.8 mm /April 25. 26 and 29). On May had normal temperatures and heavy rainfall (99.5 mm/May 4, 10, 24 and 31). June recorded high temperatures (above 30°C) and heavy rainfall due to storms (99.3 mm /June 4, 8, 13, 20 and 28). The months of July and August had no specific features in terms of temperatures,but there were storms with high precipitation (99.5 mm/ July 2, 13

and 24; 99.0 mm / August 6 and 26). The statistical analysis of the wines concerned the following biochemical indicators: alcoholic concentration (% vol), total acidity (g/L tartaric acid), dry extract (g/L), reducing sugars (g/L), the content in polyphenols (mg GAE/L) and their antioxidant activity (mg GAE/L).

Table 2 shows the correlations between the indicators taken into study. We can highlight the following items:

- The alcoholic concentration correlates positively, significantly distinct, with the total extract (r=0.628 **) and negative with density, total acidity and volatile acidity (r =-0.284, r = -0.298,r= -0.088).

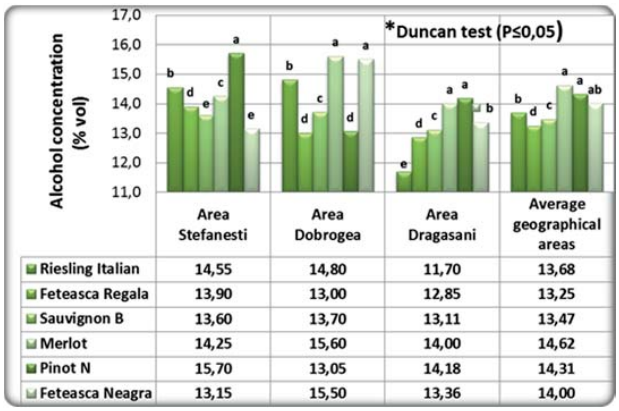
Table 2. Matrix of "r" correlation (Pearson correlation coefficients "r") of the main physical and biochemical indicators (average for the 6 wine cultivars from the 3 vineyard centers).

Indicators		Alchool streight	Dry extract g/L	Dens at 20 degrees C	Tot. acid g/L AT	Vol. Acid. g/L AA	Sugar g/L	Total anthocyanins content (mg/L)	Total polyphenol content (mg GA/g)	Antiox Activity mg GAE/L
Alchool Streight	Pearson Correlation	1	.628 (**)	-.284	-.298	-.088	.088	.171	.106	.260
Dry extract, g/L	Pearson Correlation	.628 (**)	1	.067	.059	-.532 (**)	-.048	.538 (**)	.253	-.089
Dens at 20 degrees C	Pearson Correlation	-.284	.067	1	-.054	.148	.814 (**)	.116	-.439 (*)	-.072
Total acidity g/L AT	Pearson Correlation	-.298	.059	-.054	1	-.145	-.281	.338	.051	-.232
Vol. acidity g/L AA	Pearson Correlation	-.088	-.532 (**)	.148	-.145	1	.477 (**)	-.196	-.084	.643 (**)
Sugar g/L	Pearson Correlation	.088	-.048	.814 (**)	-.281	.477 (**)	1	-.034	-.557 (**)	.250
Total anthocyanins content (mg/L)	Pearson Correlation	.171	.538 (**)	.116	.338	-.196	-.034	1	.474 (**)	.116
Total polyphenol content (mg GA/g)	Pearson Correlation	.106	.253	-.439 (*)	.051	-.084	-.557 (**)	.474 (**)	1	.385 (*)
Antiox. activity mg GAE/L	Pearson Correlation	.260	-.089	-.072	-.232	.643 (**)	.250	.116	.385 (*)	1

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

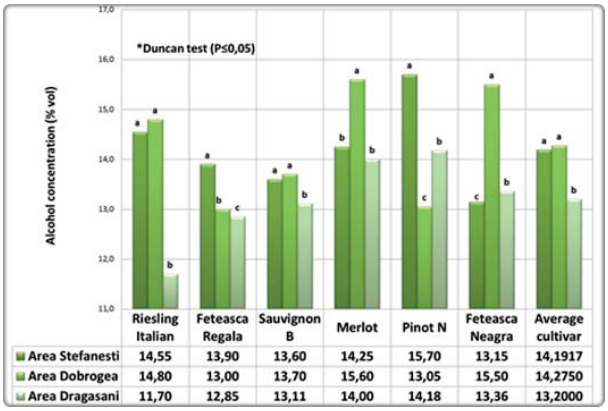
** Correlation is significant at the 0.01 level (2-tailed)

- The sugar content correlates negatively with the total acidity (r= -0.281), the dry extract (r= -0.048) and the anthocyanin content (r= -0.034) and distinctly significant with the total polyphenols (r= -0.557**).
-The pH correlates positively with the alcoholic content, volatile acidity and antioxidant activity, (r= 0.237, r=0.185, respectively r=0.064) and significant positive to sugar (r= 0.432*), which explains that there is a balanced ratio between these indicators.
-Total polyphenols from wine are correlated significantly negative to sugar (r= -0.557**) and to pH (r= -0.431*), but the anthocyanin content (r=0.474**)and antioxidant activity (r=0.385**) are distinct positively correlated with sugar.
In figure 1is presented the differences between the Dobrogea vineyard and the other two vineyards (Drăgăşani and Ştefăneşti) regarding the alcoholic strength. Concerning Fetească Neagră cultivar from Dobrogea vineyard,the alcoholic strength was around 15.5% vol., from Ştefăneşti it reached13.15% vol.and from Drăgăşani vineyard was13.36% vol. The lowest alcoholic concentrations were found in white wines, especially in Fetească Regală cultivar from Dobrogea vineyard, with 13.0% vol. and from the Drăgăşani vineyard with 12.85% vol (fig 1). Regarding the alcoholic strength of the six analysed cultivars in the three geographical areas, the highest alcohol content was recorded at the Pinot Noir wine from Ştefăneşti vineyard centre (fig 2).



	Area Stefanesti	Area Dobrogea	Area Dragasani	Average geographical areas
Riesling Italian	14,55	14,80	11,70	13,68
Feteasca Regala	13,90	13,00	12,85	13,25
Sauvignon B	13,60	13,70	13,11	13,47
Merlot	14,25	15,60	14,00	14,62
Pinot N	15,70	13,05	14,18	14,31
Feteasca Neagra	13,15	15,50	13,36	14,00

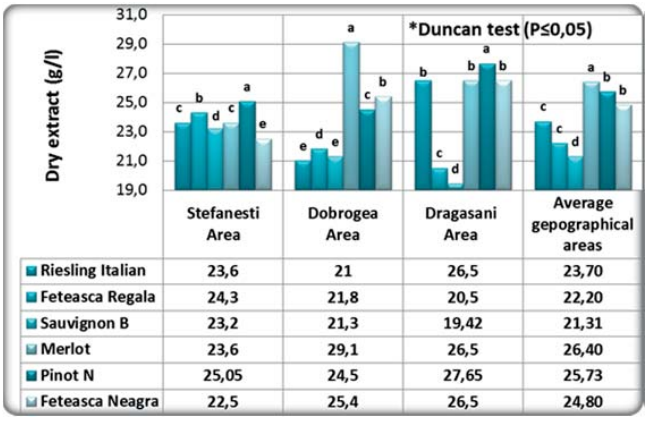
Figure 1. Influence of the cultivar on the alcoholic concentration of wine, depending on the geographical area



	Riesling Italian	Feteasca Regala	Sauvignon B	Merlot	Pinot N	Feteasca Neagra	Average cultivar
Area Stefanesti	14,55	13,90	13,60	14,25	15,70	13,15	14,1917
Area Dobrogea	14,80	13,00	13,70	15,60	13,05	15,50	14,2750
Area Dragasani	11,70	12,85	13,11	14,00	14,18	13,36	13,2000

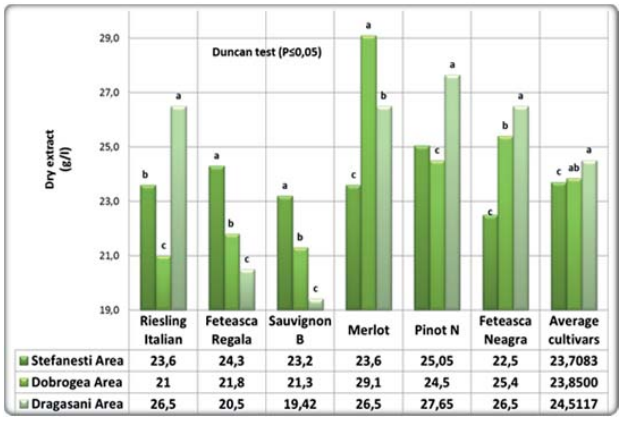
Figure 2. Influence of the geographical area on the alcoholic concentration of wine, depending on the cultivar

Figures 3 and 4 show us the influence of the area on the dry extract of wines. Consideringthe average value of the studied cultivars, it can be observed that the highest value of extracts were recorded at Drăgăşani (Merlot = 29.1g/L) and Dobrogea (Pinot Noir=27.65g/L) vineyards, and the lowest at Ştefăneşti vineyard centre.
The differences of total acidity depend on the pedological composition of the soil in each vineyard and for each cultivar. The lowest value of the average total acidity (5.21g/L) was observed at the cultivars from Dobrogea vineyard (fig. 5, 6).
The pH of wine depends on the cultivar and on the geographical area. For example for Sauvignon blanc from Ştefăneşti it was 3.92 pH and for Drăgăşani it was 3.05 pH (Fig. 7). Considering the average of the cultivars, the highest pH was recorded to the Ştefăneşti vineyard centre (3.56 pH) (fig. 8).



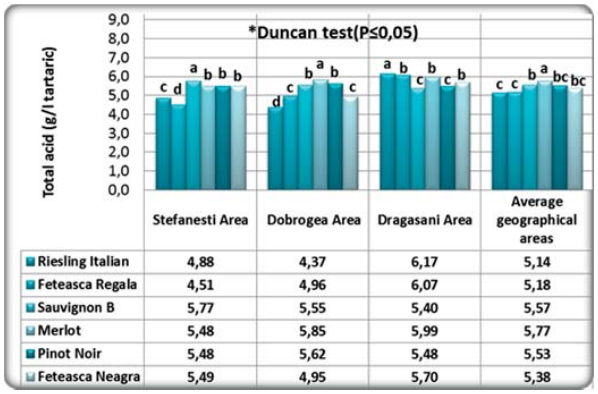
	Stefanesti Area	Dobrogea Area	Dragasani Area	Average gepographical areas
Riesling Italian	23,6	21	26,5	23,70
Feteasca Regala	24,3	21,8	20,5	22,20
Sauvignon B	23,2	21,3	19,42	21,31
Merlot	23,6	29,1	26,5	26,40
Pinot N	25,05	24,5	27,65	25,73
Feteasca Neagra	22,5	25,4	26,5	24,80

Figure 3. Influence of the cultivar on the dry extract of wine, depending on the geographical area



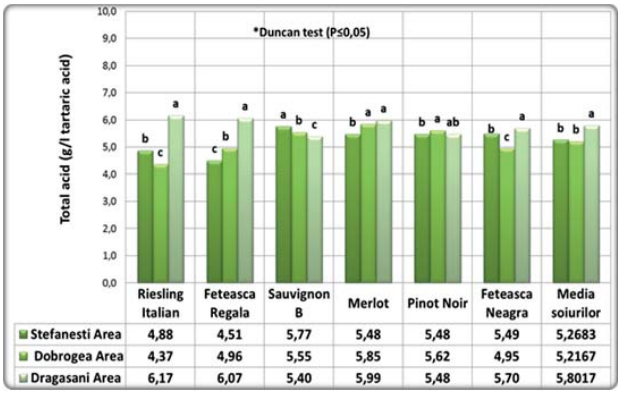
	Riesling Italian	Feteasca Regala	Sauvignon B	Merlot	Pinot N	Feteasca Neagra	Average cultivars
Stefanesti Area	23,6	24,3	23,2	23,6	25,05	22,5	23,7083
Dobrogea Area	21	21,8	21,3	29,1	24,5	25,4	23,8500
Dragasani Area	26,5	20,5	19,42	26,5	27,65	26,5	24,5117

Figure 4. Influence of the geographical area on the dry extract of wine, depending on the cultivar



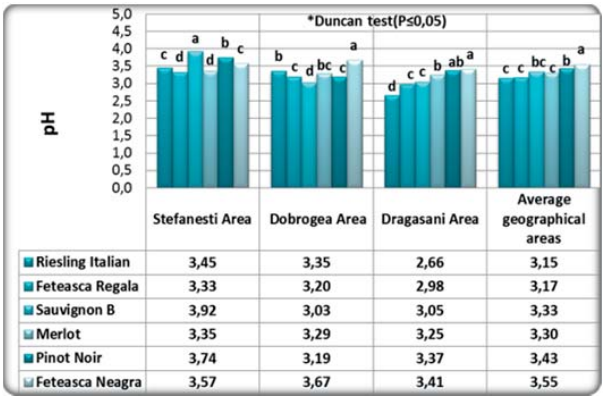
	Stefanesti Area	Dobrogea Area	Dragasani Area	Average geographical areas
Riesling Italian	4,88	4,37	6,17	5,14
Feteasca Regala	4,51	4,96	6,07	5,18
Sauvignon B	5,77	5,55	5,40	5,57
Merlot	5,48	5,85	5,99	5,77
Pinot Noir	5,48	5,62	5,48	5,53
Feteasca Neagra	5,49	4,95	5,70	5,38

Figure 5. Influence of the cultivar on the total acidity, depending on the geographical area



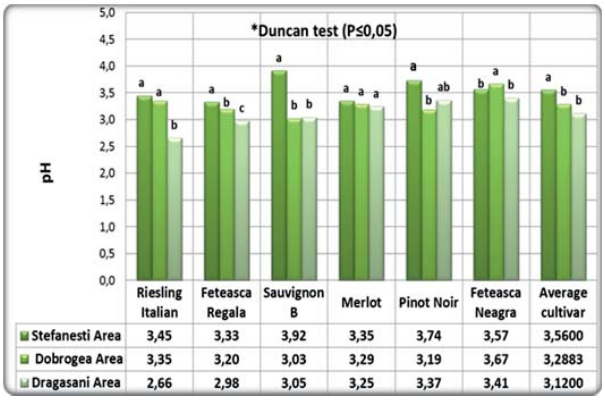
	Riesling Italian	Feteasca Regala	Sauvignon B	Merlot	Pinot Noir	Feteasca Neagra	Media soiurilor
Stefanesti Area	4,88	4,51	5,77	5,48	5,48	5,49	5,2683
Dobrogea Area	4,37	4,96	5,55	5,85	5,62	4,95	5,2167
Dragasani Area	6,17	6,07	5,40	5,99	5,48	5,70	5,8017

Figure 6. Influence of the geographical area on the total acidity,depending on the cultivar



	Stefanesti Area	Dobrogea Area	Dragasani Area	Average geographical areas
Riesling Italian	3,45	3,35	2,66	3,15
Feteasca Regala	3,33	3,20	2,98	3,17
Sauvignon B	3,92	3,03	3,05	3,33
Merlot	3,35	3,29	3,25	3,30
Pinot Noir	3,74	3,19	3,37	3,43
Feteasca Neagra	3,57	3,67	3,41	3,55

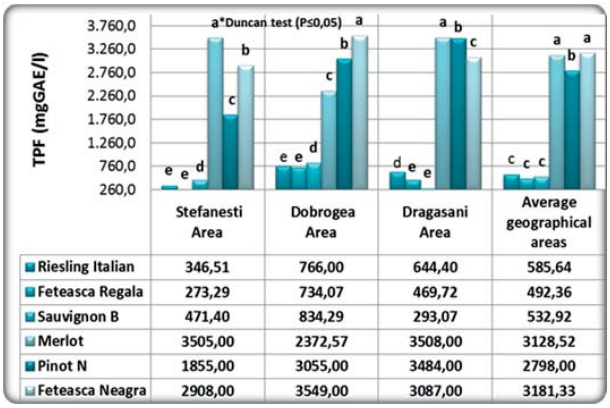
Figure 7. Influence of the cultivar on the pH of wine, depending on the geographical area



	Riesling Italian	Feteasca Regala	Sauvignon B	Merlot	Pinot Noir	Feteasca Neagra	Average cultivar
Stefanesti Area	3,45	3,33	3,92	3,35	3,74	3,57	3,5600
Dobrogea Area	3,35	3,20	3,03	3,29	3,19	3,67	3,2883
Dragasani Area	2,66	2,98	3,05	3,25	3,37	3,41	3,1200

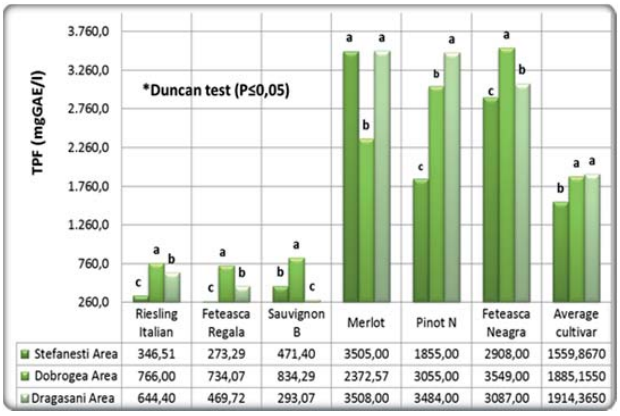
Figure 8. Influence of the geographical area on the pH of wine, depending on the cultivar

In figures 9 and 10 is showed the influence of the geographical area on the content of total polyphenols in wine, depending on the cultivar. For the red wines, the highest content of total polyphenols was recorded at Fetească Neagră (3549 mg GAE/L) from Dobrogea vineyard, while for Pinot Noir from Ştefăneşti vineyard center was recorded the lowest total polyphenols content (1846 mg GAE/L).



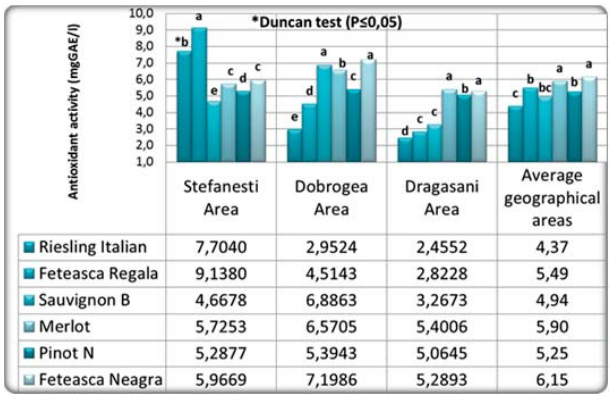
	Stefanesti Area	Dobrogea Area	Dragasani Area	Average geographical areas
Riesling Italian	346,51	766,00	644,40	585,64
Feteasca Regala	273,29	734,07	469,72	492,36
Sauvignon B	471,40	834,29	293,07	532,92
Merlot	3505,00	2372,57	3508,00	3128,52
Pinot N	1855,00	3055,00	3484,00	2798,00
Feteasca Neagra	2908,00	3549,00	3087,00	3181,33

Figure 9. Influence of the cultivar on the content of total polyphenols, depending on the geographical area



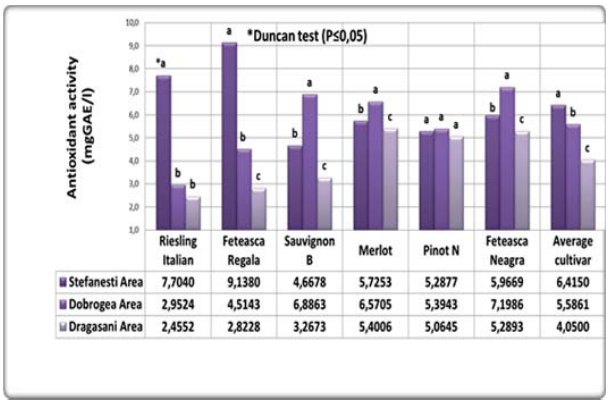
	Riesling Italian	Feteasca Regala	Sauvignon B	Merlot	Pinot N	Feteasca Neagra	Average cultivar
Stefanesti Area	346,51	273,29	471,40	3505,00	1855,00	2908,00	1559,8670
Dobrogea Area	766,00	734,07	834,29	2372,57	3055,00	3549,00	1885,1550
Dragasani Area	644,40	469,72	293,07	3508,00	3484,00	3087,00	1914,3650

Figure 10. Influence of the geographical area on the content of total polyphenols, depending on the cultivar



	Stefanesti Area	Dobrogea Area	Dragasani Area	Average geographical areas
Riesling Italian	7,7040	2,9524	2,4552	4,37
Feteasca Regala	9,1380	4,5143	2,8228	5,49
Sauvignon B	4,6678	6,8863	3,2673	4,94
Merlot	5,7253	6,5705	5,4006	5,90
Pinot N	5,2877	5,3943	5,0645	5,25
Feteasca Neagra	5,9669	7,1986	5,2893	6,15

Figure 11. Influence of the cultivar on antioxidant activity, depending on the geographical area



	Riesling Italian	Feteasca Regala	Sauvignon B	Merlot	Pinot N	Feteasca Neagra	Average cultivar
Stefanesti Area	7,7040	9,1380	4,6678	5,7253	5,2877	5,9669	6,4150
Dobrogea Area	2,9524	4,5143	6,8863	6,5705	5,3943	7,1986	5,5861
Dragasani Area	2,4552	2,8228	3,2673	5,4006	5,0645	5,2893	4,0500

Figure 12. Influence of the geographical area on the antioxidant activity, depending on the cultivar

Antioxidant activity was influenced by the climate and pedological characteristics. The antioxidant activity of the wines is directly proportional to the concentration of total polyphenols in wine. The cultivar with the highest antioxidant activity was Fetească Neagră (1.6582mgGAE/l) from the Dobrogea vineyard (fig. 12). For all the studied cultivars the highest values of this chemical parameterwere recorded at Dobrogea vineyard.

CONCLUSIONS

- The experimental data showed differences concerning the alcohol concentration for the same cultivar due to the differences of soil, climatic conditions and winemaking technology.
- With regard to the alcoholic strength of the sixcultivars analysed in the three geographical areas, the highest alcohol content was recorded for Pinot Noir wine in Ştefăneşti vineyard centre.
- Among the correlations between the studied indicators we highlight the following: the alcoholic concentration correlates positively, significantly distinct with the total extract (r=0.628 **) and negative with total acidity and volatile acidity (r = -0.298,r= -0.088); the dry extract of the wines correlates negatively (distinctly significant) with the volatile acidity (r= -0.538**) and negative with antioxidant activity (r= -0.089); the total polyphenols from

wine are significantly negative correlated to sugar (r= -0.557**) and to pH (r= -0.431*), but to the anthocyanin content (r=0.474**)and antioxidant activity (r=0.385**) are distinctly positive correlated.

- Among the studied wines, the wines with high alcoholic concentrations are from Dobrogea vineyard (Merlot 15.6%vol. Riesling 14.8%vol), and the wines with the lowest alcoholic concentrations are from Drăgăşani vineyard (Riesling 11.3% vol, Fetească Regală 12.85%vol).
- Antioxidant activity was influenced by the climate and pedological characteristics. The antioxidant activity of the wines is directly proportional to the concentration of total polyphenols in the wine. The cultivar with the highest antioxidant activity was Fetească Neagră (1.6582mgGAE/L) from Dobrogea vineyard. For all the studied cultivars, the highest values of this chemical parameterwas recorded at Dobrogea vineyard.

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THE INFLUENCE OF HAIL AND TREATMENTS WITH AMINO-ACIDS ON THE FERTILITY AND BIOLOGIC BALANCE OF GRAPEVINES

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ABSTRACT

Climatic accidents increase in frequency due to climatic changes. Hailstorms are typically localized events and are known few studies about their effect on grapevine. The objective of this study was to examine the vine vegetative balance responses to natural hail, registered when shoots were 10-15 cm height. Four grape cultivars (*Vitis vinifera* L.), two for red wine (Fetească Neagră, Pinot Noir) and two for white wine (Chardonnay, Tămâioasă românească) were studied in Dealu Mare, one of the most known vineyards in Romania. Visibly, the extent of the hailstorm damage was great, enough to injure or remove about 90% of shoots growth till hail fall. Three treatments with Delfan Plus (amino-acids complex) were applied on grapevine canopy. Number of shoots/vine and number of fertile shoots/vine were counted and biologic balance of grapevine indexes were calculated. Our results indicated that natural hail affect significantly the number of fertile shoots. Delfan Plus treatments positively influenced the number of shoots and fertile shoots. Delfan Plus treatments had little influence on Recovery index, vine Balance indexes and Ensurance Coefficient after one single year of study. The capacity of recovery after hail fall, in this case, is more influenced by the grape cultivar. The present work is the first attempt to enhance our understanding on the vegetative responses of grapevines to the use of biostimulants and to natural hail, which is an extreme and complex natural phenomenon.

Keywords: grapevine, Delfan Plus, fertile shoots, self-regeneration index, ensurance coefficient

INTRODUCTION

Hail represents an important climatic risk, although it occurs quite rarely. In a short time it can cause major material damage in the vineyards, having a direct impact on growth,

harvest and last, but not the least, on the quality of grapes as raw material (Bora et al., 2014; Bora et al., 2016; Stafne and Carroll, 2019). Hail falls can have a destructive effect on vineyards, depending on the intensity, size and timing of the fall, causing significant damage, compromising grape production in that year, even influencing next year's production (Teodor, 2018). In Romania, the highest frequency of hail falls is found in the hills areas of the country, where the average number of hail days per year can reach up to 11.8 days/year (northwestern Romania). In the viticultural region of the hills of Muntenia and Oltenia, the average number of days with extreme phenomena represented by the hail fall is 0.3-0.8 days/year (Burcea and Cică, 2016). In wine regions, hail can occur throughout the year, but with a very low frequency in the cold season, while the period April-September representing 94% of the total average monthly hail days, reach its maximum in summer, more precisely in July (Istrate et al., 2015). The use of biostimulants could ensure food security with a limited increase in agricultural land, a sustainable strategy is to increase plant resistance and resilience to counteract climate change-induced stresses (Calvo et al., 2014). Biostimulants have a low toxicity for the long term, and are less prone to select resistans strains of pests and pathogens, and, therefore, can be considered environment and human friendly. The biostimulants market has increased in the last two decades, and will range around USD 1.5-2 billion in 2022 only in Europe (EBIC). These biostimulants, along with the improvement of plant growth and production, also are helpful to improve the yield and yield nutritional quality when applied exogenously through different modes (Ali et al., 2020). Petcu et al, 2007, certifies that the use of fertilizers containing amino-acids, ensures an easier transition to plant stressors, caused by extreme events such as: low temperatures, hail, water stress. Even though several studies have been conducted on the effect of extreme weather events on grapevine growth and physiology (Călugăr et al., 2009; Keller et al., 2016), as well as on grape and wine quality (Greer and Weedon, 2013; Bal et al., 2014), it is not clear yet what effects hailstorms have, directly or indirectly, on grapevine performance to recover its canopy. Due to this reason, we studied the effects of natural hail on some important cultivars in Romania (Tămâioasă românească, Fetească neagră, Pinot Noir, Chardonnay) establishing its impact on vegetative growth and vine balance.

MATERIALS AND METHODS

The research was carried out during 2019 and 2020, on four *Vitis vinifera* L. cultivars – Tămâioasă românească, Fetească neagră, Pinot Noir and Chardonnay, at Serve winery, Ceptura wine growing center, Dealu Mare vineyard. The vines were plantated as follow: Pinot Noir in 2003, Fetească neagră and Chardonnay in 2006 and Tămâioasă românească in 2016. Planting distance was 2.2 m between rows and 0.9 m between vines/row, with a density of 5050 vines/ha. The vines were trained as simple Guyot (1 canes – 8 – 10 buds and 1 spur/vine) or double Guyot (2 or 3 canes – 9 -10 buds and 2 spurs/vine) and pruned as follow:

Chardonnay – simple Guyot – 2019 – 12 buds/vine; 2020 – 13 buds/vine;
Tămâioasă românească – simple Guyot - 2019 – 11 buds/vine; 2020 - 13 buds/vine;
Pinot Noir - simple Guyot - 2019 – 11 buds/vine; 2020 - 13 buds/vine.
Fetească Neagră – double Guyot - 2019 – 24 buds/vine; 2020 – 25 buds/vine.

On April 30th 2019, the hail that felt in Ceptura wine growing center, damaged 90 % of shoots. The shoots were 10-15 cm height, uniformly growth and in good phitosanitary shape (Figure 1). In 30 minutes felt an amount of 25 mm/m^2 of rain. On May 2nd, at 2 days after the hail felt, a contact funcigide (Funguran OH, 2 l/ha) was applied (Figure 2). The damaged shoots were cut down to stimulate the growth of dormant, secondary and coronary buds (Figure 3). Treatments with amino-acids (Delfan Plus) were applied at 20-25 cm shoots

lenght, at 35-40 cm shoots lenght and at berries growth (treated variant) (Figure 4). Also, during vegetative period, nine phitosanitary treatments using contact and systemic pesticides were applied. After 4 weeks, the new shoots (from secondary and dormant buds) on 30 control vines and on 30 Deflan Plus treated vines were counted. During dormancy, the canes of vines were also counted and vegetative indexes were calculated as follow:
Recovery index (RI) = no. of canes from multiannual wood/no. of replaceble spurs at pruning (RI = 1- vine balance; RI <1- low vigor; RI>1- high vigor).
Balance index = no. of fertil canes /no. of two years canes (BI = 1 - vine balance; BI < 1 – low vigour; BI > 1- high vigor)
Ensurance coefficient = Recovery index + Balance index (EC = 2- vine balance; EC < 2 - low vigour; EC > 2 – high vigor).
Statistical Analysis. Number of shoots and number of fertile shoots and the influence of treatment and year on those parameters was evaluated by statistical methods using the Duncan test ($p \leq 0.05$), SPSS Version 24 (SPSS Inc., Chicago, IL., USA).

RESULTS AND DISCUSSIONS

According to Irimia et al. (2017), in the last three decades in the viticultural areas of Muntenia and Oltenia were recorded the following climatic conditions: the sum of active temperature 3192°C, average temperature in the growing season 17.7°C, precipitation level 392 mm, sunshine duration 1509 hours and a mean oenoclimatic aptitude index of 4599. This climatic data, reveals the suitability of this region for growing red and white wine cultivars.

Figure 1. Shoots before hail fall

Figure 2. Shoots after hail fall, 90 % damage

At 10-14 days after the hail fall, the growth of the shoots was achieved by dividing the cells and elongating the preformed internodes in the bud phase, by the energy stored at the cellular level. Subsequently, the development of the shoot intensified due to the entry into activity of the intercalary meristems, of the internodes and the formation of new vegetative growths that under the influence of photosynthesis, determined the formation of a rich foliar canopy (Poni et al., 2009).

Simultaneously with the growth of the shoots, there was also the growth of the afferent organs (leaves, lateral shoots, inflorescences, grapes) and the differentiation of the fruit elements for next year.

Figure 3. Vine recovery – shoots from secondary buds

Regarding the influence of hail on the vegetative organs, the lack of bud load stimulated significant increases of the foliar apparatus. The number of shoots/vine was slightly influenced, depending on the cultivar and the variant treated with amino-acids.

Figure 4. Moments of treatments with Delfan Plus, during 2019
(left – 2 weeks after hail, centered – 3 weeks after hail, right - 12 weeks after hail)

As it may be seen in Figure 5, the number of shoots on vines were close for Chardonnay, Pinot Noir and Tămâioasă românească cultivars in both variants and both years. Although, there are some significant difference between the variants at Tămâioasă românească, with 13.97 shoots/vine on Delfan Plus treatement and 12.50 shoots/vine for Control, in 2019. An interesting situation for this cultivar is that, the following year, the Control recorded higher number of shoots (13.88 shoots/vine) compared with treated variant (11.47 shoots/vine). Fetească Neagră registered the most significant difference between the studied year regarding the number of shoots per vines, but no influence between variants (treated and control) (Figure 5).

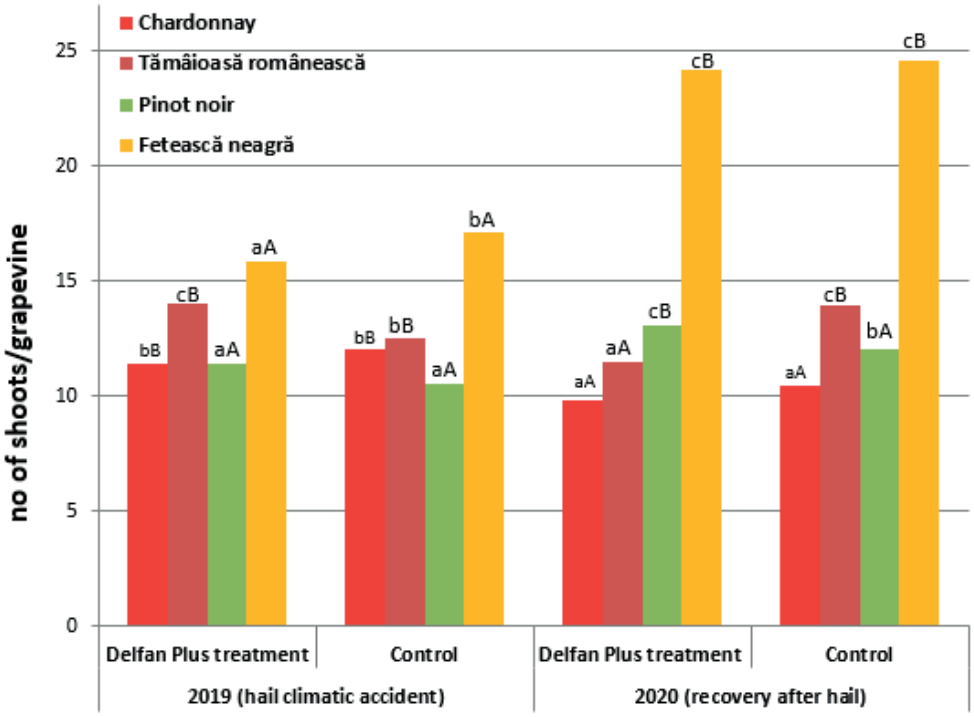

Figure 5. The number of shoots on grapevine for the two variants (Control and Delfan Plus treatment)

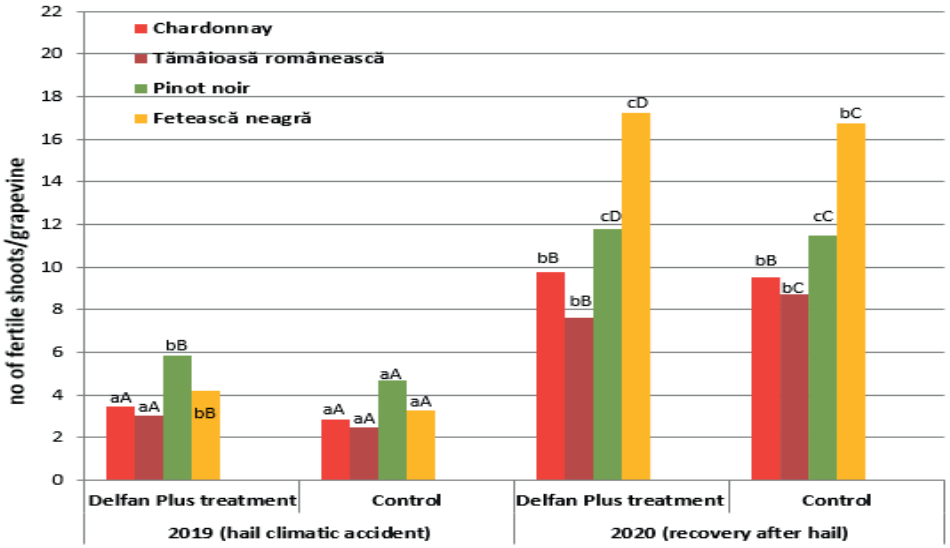

Figure 6. The number of fertile shoots on grapevine, on Delfan Plus treated variant and Control, during 2019 and 2020

Lowercase letters represent the significance of the cultivar difference between traeted and control varints ($p \leq 0.05$). Uppercase letters represent the significance of the same cultivar in the two years ($p \leq 0.05$). The difference between any two values, followed by at least one common letter, is insignificant.

The fertility of the cultivar represents its property to generate and form fruitage organs, the level of fruitage being different from one cultivar to another, to the same cultivar from one vine to another, from one year to another (Candolfi-Vasconcelos and Koblet, 1990).

The low percentage of fertile shoots on the stem and a small number of inflorescences on the stem is directly influenced by the damage caused by hail. The average number of fertile shoots differs significantly for all cultivars observed in the two years of research (2019 and 2020), but also between Treated and Control variants.

It is very clearly from Figure 6., that hail have an important influence on the number of fertile shoots, with the lower values for both variants (Treated and Control) for all the studied cultivars.

Chardonnay and Tămâioasă românească cultivars, recorded similiar fertile shoots number for variant treated with Delfan Plus and Control in the year 2019, after hail fall. In 2020, significant differences between variants (treated and control) were recorded for Tămâioasă românească, Pinot Noir and Fetească Neagră, with higher number of fertile shoots for Delfan Plus treated variant.

The share of fertile shoots or unfertile shoots (suckers) on vine depending on the cultivar is represented in the Figure 7. The values were divided and compared according to the Treatments performed with amino-acids and Control.

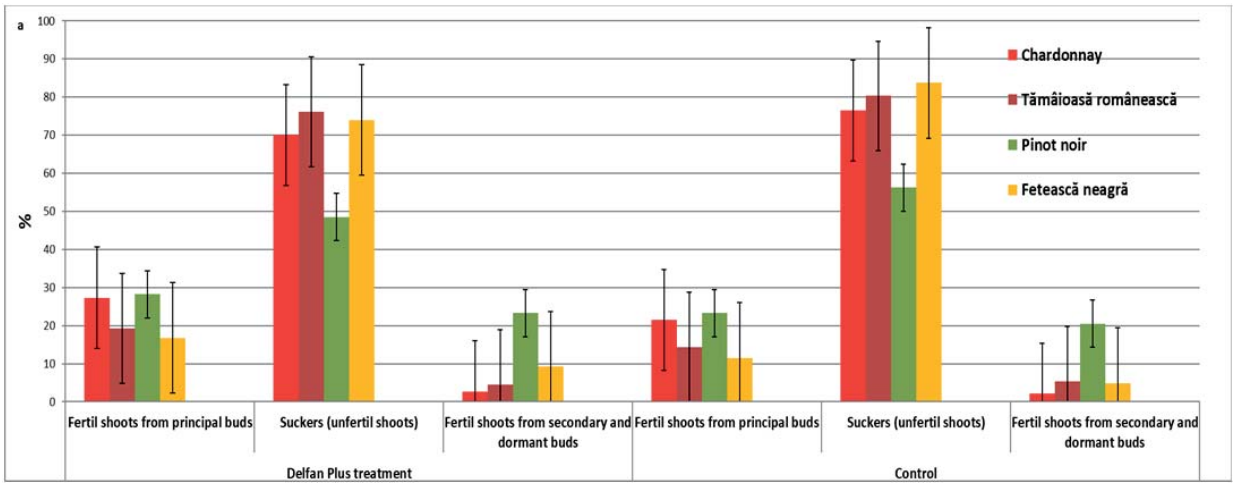

Figure 7. The percentage of fertile shoots, unfertile shoots (suckers) and fertile shoots from secondary and dormant buds, on grapevines, in 2019 period of vegetation

For all the studied cultivars, the percentage of fertile shoots from principal buds and fertile shoots from secondary and dormant buds were higher for Treated variants compared to Control. After treatment with Delfan Plus, Chardonnay recorded 27 % fertile shoots from principal buds and 21% shoots from secondary and dormant buds, values higher compared to Control, 21% and 2,1 %, respectively. Similar results were observed for Pinot Noir (Figure 7). The percentage of fertile shoots, as an index of fertility and production, is of great interest in the choice of vine cultivars and in the profitability of plantations (Candolfi-Vasconcelos and Koblet, 1990). It varies depending on the fertility of the cultivar but also on other factors.As previously observed by Petoumenou et al., 2019, lateral shoots could provide assimilates to support their own growth and sending the surplus to the main shoot, which contributed to harvest rippening.

The evaluation of the vegetative biological balance of the vines is performed based on the Recovery Index (RI), Balance Index (BI) and Ensurance Coefficient (EC). With the help of these indices, one can appreciate the accuracy of the initial bud load established last year and the pruning correction of the current year. The vegetative biological balance exists when the aerial part and the root system satisfy each other and fully meet the need for

metabolic substances, and the biomass production of the trunk is maintained in balance year after year (Irimia, 2006).

Among grapevine cultivars, the highest values of Self-regeneration index were obtained for Tămâioasă Românească (10.6 – Delfan Plus treatment and 10.0 - Control), followed by Fetească Neagră cultivar (9.7 - Delfan Plus treatment and 11.4 – Control). The lowest results were obtained for the Pinot Noir cultivar (5.5.- Delfan Plus treatment and 5.9 – Control). As it may seen from Table 3, the values of vegetative balance index of grapevine have close values between treated and control variant. This fact could indicate that Delfan Plus treatments had no significant influence on those indexes, after one single year of study. The capacity of recovery after hail fall, in this case, is more influenced by the grape cultivar (Poni et al., 2006). Also, as it was showed in previous results, that Delfan Plus treatment, have influnced the fertility of shoots for the following vintage year.

The high values, both of the Balance Index (BI) and of the Recovery Index (RI) in 2019 for all the cultivars, reflect the tendency of the vines to recover their aerial part (shoots started in vegetation) lost due to hail fall, based on dormant and secondary buds. From those buds, arise unfertile and fertile shoots, which generate the accumulation of substances necessary for the development of the root system, then sustaining in the following year (2020) a much higher bud load.

Table 1. The indexes of vegetative balance of grapevine, after hail fall and treatments with Delfan Plus

	Recovery Index		**Balance Index**		**Ensurance Coefficient**	
	Control	Delfan Plus treatment	Control	Delfan Plus treatment	Control	Delfan Plus treatment
Chardonnay	9,2	8,0	2,0	2,0	11,2	10,0
Tămâioasă românească	10,0	10,6	2,0	2,0	12,0	12,6
Pinot Noir	5,9	5,5	2,0	2,0	7,9	7,5
Fetească Neagră	11,4	9,7	2,0	2,0	13,4	11,7

Although large work has been done to study the role of biostimulants in improving plant growth and production, still more is required for final recommendations. More attention is required in this regard for the future, due to their non-toxic and environmental-friendly nature.

CONCLUSIONS

The present study is the first attempt in quantifying natural hail effects on vegetative balance on some wine grapes cultivars. Based on the results, the natural hailstorm caused an alteration in vegetative growth on Chardonnay, Pinot Noir, Tămâioasa românească and Fetească Neagră (*V. vinifera* L.) grapevines due to shoots damage induced by the hailstorm. The treatments with biostimulators such as Delfan Plus after hail and the following year, help grapevine to recover vegetative canopy with significant increases compared with control variant (wihtout treatment). Moreover, this phenomenon implicates physiological and vegetative responses that can bring the vines to an acceptable vine balance index and bud fertility in the following season. Further investigation of these effects (maybe in the presence of greater hail-damage impacts) could be useful for better understanding the responses of grapevines under extreme abiotic conditions.

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SELECTION OF AUTOCHTHONOUS *SACCHAROMYCES* AND NON-*SACCHAROMYCES* YEASTS STRAINS ACCORDING TO THEIR EXTRACELLULAR ENZIMATIC ACTIVITY

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ABSTRACT

The aim of the present study was to investigate the production of extracellular enzymes in a number of twenty six autochtonous *Saccharomyces* and non-*Saccharomyces* strains selected in Dealu Mare region for wine production. The strains were screened for the production of extracellular b-glucosidase, esterase, pectinase and protease activity by inoculation the yeast strains onto selective media. All *Saccharomyces* tested strains showed at least two enzymatic activities while non-*Saccharomyces* strains showed activity at least for one enzyme. The weakest activity was recorded in case of β-glucosidase. Most of the tested strains exhibit more or less intense activity for polygalacturonase/pectinase and protease. This study put into evidence the potential of autochtonous and especially of non-*Saccharomyces* strains as source of production of secondary compounds which can play an important role in improving the quality of wines.

Keywords: fermentation, enzymatic activity, selection, Saccharomyces, non-Saccharomyces.

INTRODUCTION

In recent years, there has been growing the interest in using autochthonous *Saccharomyces* and non-*Saccharomyces* strains in controlled double or multistarter cultures to improve wine quality (Strauss et al., 2001; Jolly et al., 2014; Dutraive et al., 2019).Several studies have pointed out that non–*Saccharomyces* yeasts produce and secrete several enzymes (esterases, glycosidases, lipases, b-glucosidases, proteases, cellulases, etc.) that could have a positive influence on the characteristics of the wine, mainly on the varietal aroma (Charoenchai et al., 1997). In Romania, until now, there has been a real interest and results in the isolation and oenological characterization only of yeast strains belonging to the genus *Saccharomyces*, there are no concerns regarding the screening of non-*Saccharomyces* species, as well as the use of mixed cultures in the vinification process. The objective of this study was to evaluate the extracellular enzymatic activity of 26 autochthonous *Saccharomyces* and non-*Saccharomyces* strains selected in Dealu Mare region for wine production. On the basis of the results, the best strains will be used in double or sequential culture aiming to improve the characteristics of resulted wines.

MATERIALS AND METHODS

Twenty six yeast strains belonging to*Saccharomyces*, *Candida* and *Debaryomyces* genus were screened for the production of extracellular b-glucosidase, esterase, pectinase and protease

activity. All strains were isolated and selected from the vineyard of Valea Călugărească viticultural centre, from the grape surface and from various phases of must fermentations and identified by means of conventional morphological, physiological and biochemical procedures and also through molecular biology analysis (data reported elsewhere). Cultures were maintained in the microorganisms collection of the Research Institute for Viticulture and Oenology Valea Călugărească on Yeast Extract Peptone Glucose agar medium, on slants under paraffin oil and subcultivated every 6 months on the same medium. In order to screen the selected wine yeasts for the extracellular enzymatic activity the yeast strains were inoculated onto selective media. The screening for β-glucosidase activity was perform by inoculation of the yeast strains on medium containing (g/L): Yeast Nitrogen Base, 6.7; cellobiose, as carbon source 10 and agar, 20. pH of the medium was adjusted at 5.5 (Fernanda Gaensly et al., 2015).
Extracellular esterase activity was determined by using a medium with the following composition (g/L): peptone, 10; NaCl, 5; $CaCl_2x2H_2O$, 0.1; Tween 80 (polyoxyethylene sorbitan monooleate), 10 and agar, 20, pH 6.8). Yeasts with enzymatic activity hydrolyze the substrate and a precipitate (opaque halo) is visible around the colonies (Slifkin, 2000).
The protease activity assay was carried out on medium containing (g/L): yeast extract, 3; malt extract, 3; peptone, 5; glucose, 10; NaCl, 5; agar, 15. In a separate bottle, an equal volume of skimmed milk dissolved in sterile water was prepared. After sterilization, the two solutions were mixed and distributed into sterile Petri dishes. The presence of a clear zone around the inoculum indicated the protease activity (Comitini et al., 2011).
Polygalacturonase/pectinase activity was determined as described by Strauss et al. (2001) with some modifications. Yeasts were cultivated on polygalacturonate agar medium containing (g/L): polygalacturonic acid, 12.5; potassium phosphate, 6.8; Yeast Nitrogen Base, 6.7; glucose, 10; and agar, 20. After colonies growth, plates were flooded with hexadecyltrimethylammonium bromide (10 g/L). Colonies showing clear halo around were identified as positive. Yeasts suspensions after 24 h of culture (A580 = 0.5, corresponding to a cell concentration of 10^6 /ml) were used for inoculation (after P. Buzzini and A. Martini, 2002).Inoculation of the yeast strain was performed on the surface of the sterilized medium by autoclaving at 120°C for 15 min. (from place to place).The cultures were incubated at 28°C for 5-7 days. For each analyzed strain, 3 repetitions were performed. Observations were made daily on cell growth. Yeasts with enzymatic activity hydrolyze the substrate.
The assessment of the degree of production of extracellular enzymes during the vinification process was made by measuring the colonies and establishing the degree of growth after 120 hours. The interpretation of the results was done as follows (Table 1):

Table 1. Interpretation of the results concerning the degree of enzymatic activity

Degree of colony growth	Results
0 mm	No enzymatic activity
0.10 – 0.20 mm	Very low activity
0.21 – 0.30 mm	Low activity
0.31 – 0.40 mm	Intense activity
> 0.40 mm	Very intense activity

RESULTS AND DISCUSSIONS

Both *Saccharomyces* and non-*Saccharomyces* yeasts strains showed extracellular enzymatic activity for at least two enzymes (Table 2).

Table 2. Characterization of extracellular enzymatic activity of *Saccharomyces* yeasts strains

Saccharomyces cerevisiae (Code)	Extracellular enzymatic activity			
	β Glucozidase	Esterase	Protease	Pectinase
21	+	-	+++	+
23	++	++	-	-
24	++	+++	+	+
26	-	+++	++	+
28	-	+	++++	+
29	+	+++	++++	++
30	++	+++	+	+
33	++	+	-	++
34	+	+	++	++
35	+	+	++++	++
36	+	+++	++++	-
37	+	+	++++	++
75	+	++++	++++	-
76	+	++++	++++	++
77	-	+++	+	++
79	+	+++	+	++
137	-	++	+++	-

Legend: **-** no activity; + very low activity; ++ low activity; +++ intense activity; ++++ very intense activity

A similar study perform by González, J.A. et al. (2004), aiming to evaluate the variability of enzymatic activities during the wine fermentation using 15 yeast strains, revealed that all the *non-Saccharomyces* strains tested showed at least one enzymatic activity, while *Saccharomyces*strains showed only two enzimatic activities.

Very low and low activity was registered, in case of *Saccharomyces cerevisiae* strains, for the enzymes β glucosidase and pectinase, while for the esterase and protease was registered intense and very intense activity in a high percentage.

Concerning β glucosidase activity, similar results were obtained by Fia G. et al. (2005). Very weak or no detectable hydrolytic activity was observed in case of *Saccharomyces cerevisiae* strains, while non- *Saccharomyces* strains exhibited different degree of β glucosidase activity.

In a study performed by Rosi I, et al. (1994), 317 strains, representing 20 species of yeasts, were screened for the presence of β-glucosidase activity. All the strains of the species *Debaryomyces castellii, Debaryomyces hansenii, Debaryomyces polymorphus, Kloeckera apiculata* and *Hansenula anomala* showed β-glucosidase activity, but only one of the 153 strains belonging to *Saccharomyces cerevisiae.*

In our study, 13 yeasts strains representing 76.47% showed β glucosidase activity. In case of 9 strains (52.94%) the activity was very low (colony increases between 1.0 and 2.0

mm) and for 4 strains the activity was low (colony increases between 2.1 and 3.0 mm); 5 of the strains (29.41%) showed very low pectinase activity and 8 strains (47.06%) showed low activity;16 strains (94.12%) showed esterase activity, in case of 7 of them (41.18%) the activity being very intense; 15 strains (88.24%) exhibited protease activity, in case of 7 of the strains (41.18%) the activity being very intense (Table 3; Figure 1,2).

Table 3. The intensity of extracellular enzymatic activity registered by *Saccharomyces* yeasts strains

Enzyme	Positive strains		No activity		Very low activity		Low activity		Intense activity		Very intense activity	
	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
β Glucozidase	13	76.47	4	23.53	9	52.94	4	23.53	0		0	
Esterase	16	94.12	1	5.88	5	29.41	2	11.76	7	41.18	2	11.76
Protease	15	88.24	2	11.76	4	23.53	2	11.76	2	11.76	7	41.18
Pectinase	13	76.47	4	23.53	5	29.41	8	47.06				

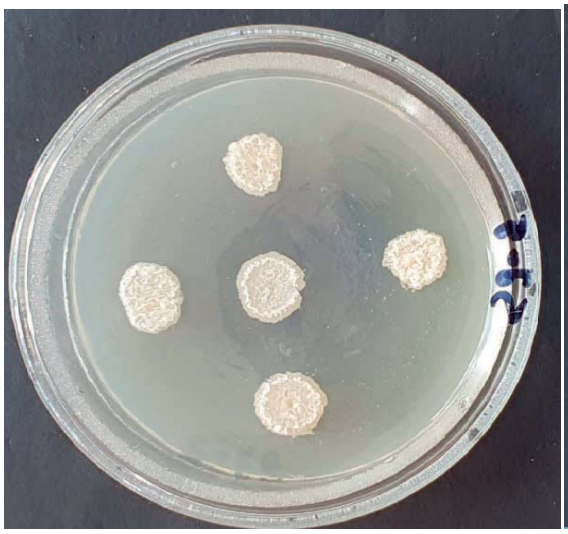

Figure 1. Strain yeast with intense esterase activity

Figure 2. Strain yeast with intense pretease activity

Most of the non-*Saccharomyces* yeasts strains taking into study showed very low and low enzyme activity with some exceptions.

Candida magnoliae strains 5 and *Debaryomyces chevallieri* strain 20b were noted by very intense protease activity, while *Candida utilis* strain 56 showed a very intense esterase activity (Table 4).

Similar results were reported by Charoenchai et al. (1997) following the researches concerning the effect of nitrogen sources on the production of extracellular proteases by non-*Saccharomyces* wine yeasts. From 26 yeast strains, protease activity was observed in strains of *Candida pulcherrima, K. apiculata* and *Pichia anomala*.

Proteolytic activity of non-*Saccharomyces* strains belonging to *Candida* and *Debaryomyces* genus was reported by Strauss M.L.A. et al. (2001).

Table 4. Characterization of extracellular enzymatic activity of non - *Saccharomyces* yeasts strains

Yeast strain (Code)	Species	Extracellulat enzymatic activity			
		β Glucozidase	Esterase	Protease	Pectinase
1	*Candida lusitaniae*	+	+	+	-
5	*Candida magnoliae*	+	+	++++	+
6	*Candida magnoliae*	-	+	+	+
7	*Candida magnoliae*	+	+	+	-
56	*Candida utilis*	+	++++	+	-
57	*Candida utilis*	+	+	++	+
136	*Candida sphaerica*	+	+	++	-
241	*Candida pelliculosa*	-	++	+	+
20 b	*Debaryomyces chevallieri*	-	++	+++	-

Legend: - no activity; + very low activity; ++ low activity; +++ intense activity; ++++ very intense activity.

On the basis of these results, the best strains will be used in double or sequential culture aiming to improve the characteristics of resulted wines, respectively to enhance the aroma and flavor properties of wines (strains which showed β Glucozidase and esterase activity), to increase juice extraction from grapes, improve wine clarification and facilitate wine filtration and stabilization of must and wine (strains which showed protease and pectinase activity).

CONCLUSION

This study put into evidence the potential of autochtonous and especially of non-*Saccharomyces* strains as source of production of secondary compounds which can play an important role in improving the quality of wines. All *Saccharomyces* tested strains showed at least two enzymatic activity while non-*Saccharomyces* strains showed activity only for one enzyme. The lowest activity was recorded in case of β-glucosidase. Most of the tested strains exhibit more or less intense activity for polygalacturonase/pectinase and protease.

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DETERMINATION OF HEAVY METALS CONCENTRATION IN WINE USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

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ABSTRACT

The determination of metals in different type of wines is of high importance for the nutritional, as well as toxic effects of these elements. The main purpose of this research is to determine the concentration of Cu, Fe, Mn, Cd, Zn and Pb in wine. The analyzed samples were wine samples obtained under micro-vinification conditions and from grapevine cultivars for the white wines (Feteasca Alba, Feteasca Regala, Babeasca Gri, Sarba) and for the red wines (Merlot, Feteasca Neagră, Cabernet Sauvignon). The highest concentrations were obtained in the cultivars of grapevine for the red wines (49.86 ± 1.32 µg/L Pb) recorded by the Feteasca Neagra cultivar and (0.23 ± 0.01 µg/L Cd) for the Merlot cultivar. In the case of Cu, Zn, Fe and Mn, the highest concentrations were recorded by the vines for white wines (532.48 ± 13.79 µg/L Cu, 1845.23 ± 32.58 µg/L Zn, 1654.98 ± 26.68 µg/L Fe, 221.32 ± 17.49 µg/L Mn) in comparison to the cultivars of vines for red wines. The conclusions obtained from the analysis of heavy metals in wines through ICP-MS are as follows: grapevine cultivars for red wine recorded the highest concentrations of Cd and Pb and the grapevine cultivars for white wines recorded the highest concentrations of Cu, Zn, Fe and Mn.Wine samples do not have a metal ion concentration higher than the maximum concentration allowed by law.The storage temperature does not affect the concentration of heavy metals, dry wine has a higher concentration of heavy metals in its composition.

Keywords: heavy metals, wine, ICP-MS, DealuBujorului, Romania

INTRODUCTION

A subject on the daily agenda for modern oenology is the presence of contaminating metals, especially of heavy metals in the wines (Bora et al., 2018). Thus, nowadays, there is a great interest in identifying the various sources leading to the presence of metals in wine, aiming to reducing the content of these metals by using various treatments permitted by the legislation in force. It is necessary to realistically know the influence of various factors of endogenous nature, such as the grape cultivar, the location of the vineyards, the soil, the climatic conditions of the year (Zinicovscaiaet al., 2017). It is also necessary to know, as much as possible, the influence of various factors of exogenous nature: conditions of harvesting, vinification technology, various enological practices applied, conditions for wines preserving (Bora et al., 2018). The wine samples were obtained under micro-

vinification conditions and were made of vine cultivars for white wines (Feteasca Alba, FeteascaRegala, BăbeascaGri, Şarba) and red wines (Merlot, Feteasca Neagra, Cabernet Sauvignon).

MATERIALS AND METHODS

Two types of wine (dry and semi-dry wine) were analyzed, as well as the influence of storage temperature (25 ° C and 5 °C) on the content of metal ions. The wine samples were obtained under micro-vinification conditions and consisted of grapevine cultivars for white wines (Feteasca Alba, FeteascaRegala, Băbeascagri, Şarba) and for red wines (Merlot, FeteascaNeagra, Cabernet Sauvignon), obtained under the ecoclimatic conditions of the years 2015 and 2016. The determination of Cu, Fe, Mn, Cd, Zn and Pb was performed using inductively coupled plasma mass spectrometry (ICP-MS). The ICP-MS calibration operation was performed using the standard XXI CertiPUR multi-element solution (Merck), and the calibration operation was performed on a daily basis. The accuracy of the used method was evaluated through repeated analysis of solutions with known concentration, the obtained results ranged between 0.8 -13.1% depending on each analyzed element. The method's recovery rate for each item ranged from 84.6 -99.8%. The wine samples preparation was made by measuring a quantity of 0.2 mL of the sample over which 8 mL of reagent (7 mL 69% HNO_3 and 1 mL H_2O_2%) is added and placed directly into the digestion vessel of the Milestone START D Microwave Digestion System, waited for 15 minutes, then digested according to the Milestone Digestion System parameters shown in Table 2. After digestion, the samples are transferred to a 50 mL volumetric flask, with 1% HNO_3 for preservation.

The detection limit (LoD) and the limit of quantification (LoQ) were calculated using the following formula LoD = 3SD/s and LoQ = 10 SD/s (SD = standard deviation, s = calibration curve). Table 1 displays the calibration conditions for the ICP-MS.

Table 1. Calibration conditions (LoD, LoQ, BEC) of ICP-MS device

Element	Correlation coefficient	LoD (µg/L)	LoQ (µg/L)	BEC (µg/L)
Cu	0.9999	0.0402	0.1339	0.237
Fe	0.9999	5.2102	17.3500	71.399
Mn	0.9999	0.0102	0.0340	0.085
Cd	0.9999	0.0202	0.0673	0.027
Zn	0.9999	0.3780	1.2587	5.401
Pb	0.9999	0.0003	0.0010	0.002

BEC = background correction.

Table 2. Digestion conditions of the Milestone START D Microwave Digestion System device

Wine					
Step	Time	Airing	Temperature (^{0}C)	Pressure (Pa)	Power (W)
I	00:10:00	-	200	-	1000
II	00:15:00	-	200	-	1000
III	00:60:00	+	35	-	0

Effective metal determination was performed using an inductively coupled plasma mass spectrometer (ICP-MS) iCAP Q from Thermo Scientific, a quadrupole model for the most efficient separation of the elements. The device was daily optimized, the carrier gas was Argon 5.0 with 99.99% purity (Messer). The statistical analysis of the results was carried out using the SPSS (Statistical Package for Social Sciences) version 24.

RESULTS AND DISCUSSIONS

As can be seen, the concentration of Cu varies within wide limits, the highest concentration was recorded in Fetească Albă (532.48 ± 13.79 μg/l) and Fetească Regală (511.21 ± 1.70 μg/L), both from the production year of 2015. Of the grapevine cultivars for the red wines, the Cabernet Sauvignon cultivar from 2015 is distinguished, with the highest concentration of Cu (496.96 ± 4.95 μg/L). The white wines record 383.08 μg/L (mean value) while the red wines record 380.02 μg/L (mean value), statistically there is no difference between the two variants. The Cu distribution was significantly influenced by the crop year and by the interaction of the cultivar factor with the crop year (Table 3).As in the case of Cu and Fe, the highest values are recorded for the vine cultivars for the white wines (Feteasca Alba 1556.80 ± 29.49 μg/L and Băbească gri 1654.98 ± 36.17 μg/L, culture year 2015), while the grapevine cultivars for the red wines recorded the lowest concentrations (Cabernet Sauvignon 996.61 ± 8.11 μg/L from 2016). The distribution of Fe was significantly influenced by the crop year and also by the interaction between the cultivar factor and crop year (Table 3). The Feteasca Regală cultivars (225.32 ± 9.15 μg/L) and Merlot (212.49 ± 2.12 μg/L), from the crop year of 2015 and respectively, 2016 recorded the highest concentrations of Mn in comparison with the cultivars of BăbeascaGri from 2016 (119.19 ± 6.89 μg/L) and Cabernet Sauvignon (115.65 ± 5.06 μg/L) from 2015, which recorded the lowest concentrations of Mn. The white wines recorded 180.73 μg/L (mean value) while red wines recorded 162.65 μg/L (mean value); from the statistical point of view, there is a significant difference between the two variants, therefore, can be said that the white wine has a higher concentration of Mn than the red one (Bora al., 2018). The distribution of Mn was significantly influenced by the crop year, by the interaction of the cultivar factor and the crop year, whereas the interaction between the cultivar and the storage temperature, of year and storage temperature had a significant influence regarding this parameter (Table 3).

In the analyzed samples of wine, the concentration of Cd is very low. Higher concentrations were recorded in the Feteasca Albă cultivars (0.09 ± 0.01 μg/L) and Merlot (0.09 ± 0.01 μg/L), followed by the Feteasca Regală and Șarba cultivars. In this case, as can be seen, the Cd concentration was extremely low, in most of the analyzed cultivars this concentration was below the limit of detection (Table 3). The Cd distribution was significantly influenced by the interaction between the cultivar factor and crop year.

Higher concentrations of Zn were identified in the Șarba cultivar in both years of culture (1845.23 ± 9.90 μg/L and 1758.54 ± 18.86 μg/L). The distribution of Zn was significantly influenced by the crop year, by the interaction of the cultivar factor with the crop year, while the interaction between cultivar and storage temperature, year and storage temperature both had a significant influence on this parameter (Table 3). The grapevine cultivars for red wines recorded the highest values, especially the FeteascaNeagracultivar (49.86 ± 1.69 μg/L) followed by the Cabernet Sauvignon cultivar (40.23 ± 3.00 μg / l). White wines recorded 6.87 μg/L (mean value), while red wines recorded 36.91 μg/L(mean value), there is a statistically significant difference between the two variants, thereby, the white wine can be said to have a higher concentration of Pb than the red one. For Băbeasca gri and Feteasca Regală, the concentration of Pb is below the detection limit. The Pb distribution was significantly influenced by the crop year, by the interaction between the cultivar factor and crop year, whereas the interaction between the cultivar and the storage temperature, year and storage temperature had a significant influence on this parameter (Table 3, 4).

As can be seen in Figure 1, the concentration of the determined heavy metals has been reported to the international law so that it can be properly ascertained if the wine samples

can be consumed without having a negative impact on the consumer. In the case of Cu, Fe, Mn, Cd, Zn and Pb concentration there were no wine samples found to exceed the maximum permitted by law. Moreover, for both Cd and Pb, the obtained results are significantly lower than the values provided by the law, and for the Cu, Fe, Mn and Zn the values were close to these limits.

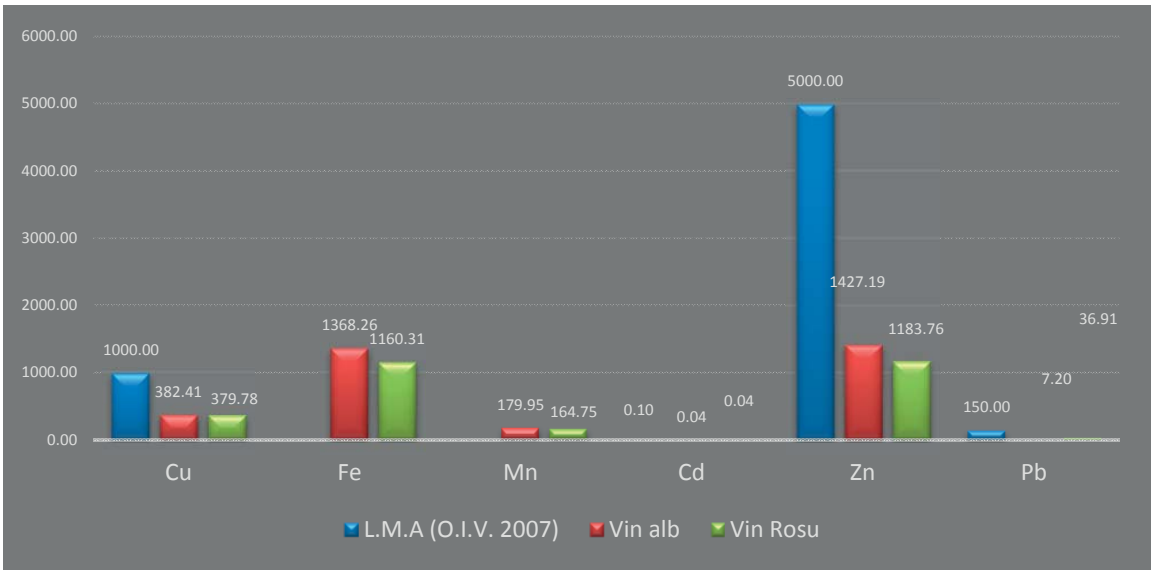

Figure 1. Reporting the obtained results to the Maximum Allowed Limits provided in O.I.V. 2007 (µg/l)

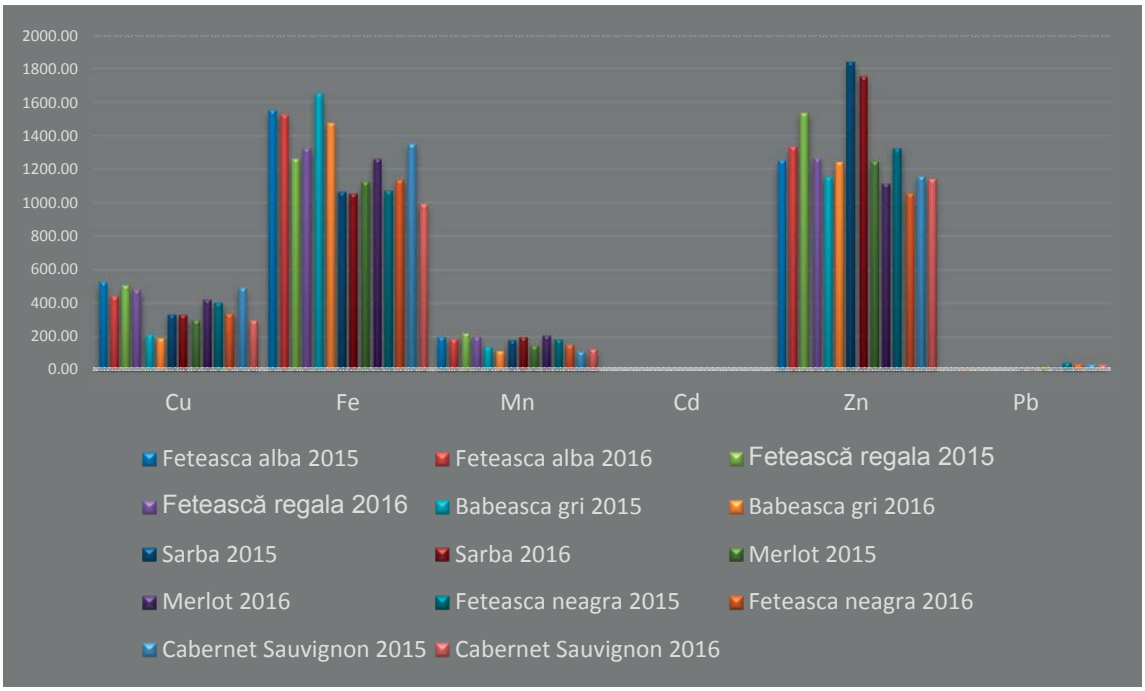

Figure 2. Influence of storage temperature (5 °C) on heavy metals concentration in wine (µg/l)

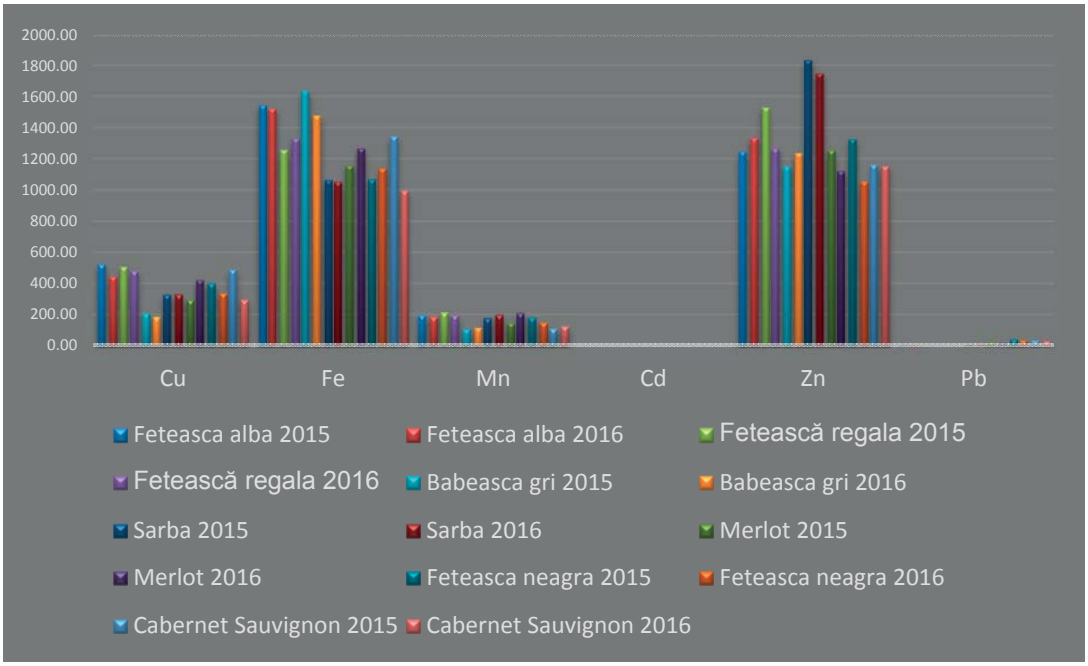

Figure 3. Influence of storage temperature (25 °C) on the concentration of heavy metals in wine (µg/l)

The storage temperature for the samples of wine had no influence on heavy metals. In both cases, their concentration was the same, with few exceptions that could be attributed to storage vessels and not to storage temperature (Figure 2 and 3).

Table 3. Concentration of heavy metals in wine.

Cultivar	Year	Storage Temp	Cu µg/l	Fe µg/l	Mn µg/l
Maximum permitted limit			**1 mg/l**	**-**	**-**
Fetească Albă	2015Semi-dry	5 ^{0}C	532.48 ± 13.79 a α	1556.80 ± 29.49 b β	203.53 ± 7.42 c β
	2016Dry		446.39 ± 13.48 d δ	1529.79 ± 7.77 b β	188.91 ± 1.18 d γ
Fetească Regală	2015Semi-dry		511.21 ± 1.70 b β	1265.78 ± 29.75 e ε	225.32 ± 9.15 a α
	2016Dry		485.32 ± 7.15 c γ	1329.49 ± 4.67d δ	204.62 ± 5.67 c β
Babească gri	2015Semi-dry		216.31 ± 5.81 iι	1654.98 ± 36.17 a α	144.24 ± 10.55 ε
	2016Semi-dry		195.39 ±5.48 j κ	1480.72 ± 8.48 c γ	119.19 ± 6.89 h η
Şarbă	2015Semi-dry		336.74 ± 7.65 g η	1069.59 ± 33.24 g η	184.42 ± 8.89 d γ
	2016Semi-dry		335.43 ± 5.59 g η	1058.91 ± 8.88 g η	203.39 ± 4.33 c β
Merlot	2015Dry		303.92 ± 6.52 h θ	1129.32 ± 9.18 f ζ	150.74 ± 2.12 ef$\delta\varepsilon$
	2016Dry		426.78 ± 5.11 e ε	1266.13 ± 47.65 e ε	212.49 ± 2.12 bcβ
Fetească Neagră	2015Dry		408.16 ± 2.60 f ζ	1077.50 ± 36.73 g η	189.10 ± 5.00 d γ

Cultivar	Year	Storage Temp	Cu µg/l	Fe µg/l	Mn µg/l
Maximum permitted limit			**1 mg/l**	**-**	**-**
	2016Semi-dry		341.30 ± 17.29 g *η*	1138.90 ± 8.13 f *ζ*	155.76 ± 8.50 e *δ*
Cabernet Sauvignon	2015Semi-dry		496.96 ± 4.95 c *γ*	1353.40 ± 5.51 d *δ*	115.65 ± 5.06 h *η*
	2016Semi-dry		301.58 ± 3.37 h *θ*	996.61 ± 8.11 h *θ*	130.04 ± 2.20 g *ζ*
Fetească Albă	2015Semi-dry		531.91 ± 2.74 a *α*	1552.07 ± 5.62 b *β*	204.35 ± 4.99 c *β*
	2016Semi-dry		450.96 ± 6.38 d *δ*	1530.15 ± 5.56 b *β*	193.25 ± 9.61 d *γ*
Fetească Regală	2015Semi-dry		513.93 ± 4.04 b *β*	1263.94 ± 14.13 e *ε*	221.66 ± 1.45 ab*α*
	2016Semi-dry		488.62 ± 7.91 c *γ*	1336.77 ± 6.80 d *δ*	203.43 ± 2.17 c *β*
Babească gri	2015Semi-dry		216.06 ± 4.24 i*ι*	1645.70 ± 19.34 a *α*	114.24 ± 5.05 h *θ*
	2016Semi-dry		193.16 ± 4.62 j *κ*	1485.46 ± 7.76 c *γ*	122.96 ± 4.57 h *ζ*
Şarbă	2015Semi-dry		336.81 ± 4.83 g *η*	1071.95 ± 2.19 g *η*	185.66 ± 1.99 d *γ*
	2016Semi-dry	25 ^{0}C	338.3.05 ± 3.05 g *η*	1059.85 ± 15.06 g *η*	206.50 ± 3.75 c *β*
Merlot	2015Dry		302.31 ± 2.76 h *θ*	1164.16 ± 65.30 f *ζ*	151.96 ± 4.56 ef*δ*
	2016Semi-dry		429.44 ± 3.70 e *ε*	1274.31 ± 7.25 e *ε*	218.57 ± 3.42 a *α*
Fetească Neagră	2015Dry		409.16 ± 1.54 f *ζ*	1075.40 ± 4.29 g *η*	189.10 ± 4.03 d *γ*
	2016Dry		344.33 ± 6.22 g *η*	1145.47 ± 5.82 f *ζ*	156.50 ± 0.61 e *δ*
Cabernet Sauvignon	2015Dry		495.20 ± 5.69 c *γ*	1350.96 ± 8.64 d *δ*	115.70 ± 4.58 h *ηθ*
	2016Semi-dry		301.14 ± 1.46 h *θ*	1002.02 ± 3.20 g *θ*	131.48 ± 6.07 g *ε*
Geana et al., 2013			500.57		223.50
Zinicovscaia et al., 2017				1100	1000
Fetească Albă	2015Semi-dry		0.09 ± 0.01 a*α*	1259.15 ± 2.70 ef*ε*	12.50 ± 1.50 g *δ*
	2016Dry		0.07 ± 0.02 abcd*αβ*	1339.22 ± 13.14 d *δ*	10.80 ± 0.68 g *δ*
Fetească Regală	2015Semi-dry		0.06 ± 0.04 d*β*	1539.93 ± 6.16 c *γ*	10.69 ± 1.44 g *δ*
	2016Dry	5 ^{0}C	UDL	1270.49 ± 21.84 e *ε*	UDL
Babească gri	2015Semi-dry		UDL	1156.75 ± 34.90 g *ζ*	UDL
	2016Semi-dry		UDL	1248.21 ± 14.74 ef*ε*	UDL
Şarbă	2015Semi-dry		UDL	1845.23 ± 9.90 a *α*	11.91 ± 0.57 g *δ*

Cultivar	Year	Storage Temp	Cu µg/l	Fe µg/l	Mn µg/l
Maximum permitted limit			**1 mg/l**	**-**	**-**
	2016Semi-dry		0.08 ± 0.02 abcα	1758.54 ± 18.86 b β	11.68 ± 0.64 g δ
Merlot	2015Dry		0.09 ± 0.01 abα	1251.24 ± 40.01 efε	29.08 ± 6.29 e γ
	2016Dry		0.09 ± 0.02 abcα	1119.11 ± 6.44 iζ	24.12 ± 5.77 f γ
Fetească Neagră	2015Dry		UDL	1328.72 ± 3.27 d δ	49.86 ± 1.69 a α
	2016Semi-dry		UDL	1058.40 ± 4.10 j η	41.20 ± 4.34 bcβ
Cabernet Sauvignon	2015Semi-dry		UDL	1161.32 ± 24.35 g ζ	40.23 ± 8.50 bcβ
	2016Semi-dry		UDL	1145.70 ± 12.29 gh$\zeta\eta$	36.98 ± 3.00 cd β
Fetească Albă	2015Semi-dry	25 ^{0}C	0.07 ± 0.02 abcd$\alpha\beta$	1254.18 ± 6.12 ef$\zeta\eta$	12.84 ± 0.93 g ζ
	2016Semi-dry		0.08 ± 0.01 abcd$\alpha\beta$	1341.86 ± 6.29 d δ	10.83 ± 0.24 g ζ
Fetească Regală	2015Semi-dry		0.06 ± 0.02 bcdβ	1536.64 ± 6.60 c γ	10.85 ± 0.24 g ζ
	2016Semi-dry		UDL	1272.90 ± 6.43 e ε	UDL
Babească gri	2015Semi-dry		UDL	1157.96 ± 6.49 g η	UDL
	2016Semi-dry		UDL	1243.45 ± 4.04 f ζ	UDL
Şarbă	2015Semi-dry		UDL	1841.59 ± 2.39 a α	11.59 ± 0.55 g ζ
	2016Semi-dry		0.07 ± 0.02 abcd$\alpha\beta$	1756.48 ± 6.78 b β	12.15 ± 0.37 g ζ
Merlot	2015Dry		0.07 ± 0.02 abcd$\alpha\beta$	1262.77 ± 3.75 ef$\varepsilon\zeta$	29.46 ± 0.90 e δ
	2016Semi-dry		0.09 ± 0.01 aα	1130.61 ± 14.62 hi θ	25.73 ± 0.51 efε
Fetească Neagră	2015Dry		UDL	1334.28 ± 7.23 d δ	49.10 ± 2.14 a α
	2016Dry		UDL	1061.52 ± 1.92 j ι	42.12 ± 0.91 b β
Cabernet Sauvignon	2015Dry		UDL	1169.08 ± 9.36 g η	40.76 ± 1.22 bcβ
	2016Semi-dry		UDL	1158.92 ± 3.33 g η	35.04 ± 2.86 d γ
Bora et al., 2018			0.12	1867.19	35.50
Zinicovscaia et al., 2017				440	

Average value ± standard deviation (n = 3). Romans' and Greek letters represent the significance of the difference (Duncan test, $p < 0.05$). The difference between any two values, followed by at least one common letter, is insignificant. M.P.L = maximal permissible limit (O.I.V., 2016). ULD = under of limit of detection. ns = insignificant

CONCLUSIONS

The experimental data shows that dry wine has the highest heavy metal content. It is also noted that these wines do not have a metal ion content higher than the maximum permitted by law. The conclusions obtained from the analysis of heavy metals in wine by ICP-MS are as follows: the grapevines cultivars for red wines have recorded the highest concentrations of Cd and Pb, and the grapevine cultivars for white wines recorded the highest concentrations of Cu, Zn, Fe and Mn; the wine samples do not have a metal ion concentration higher than the maximum permitted by law; the storage temperature does not influence the concentration of heavy metals; dry wine has a higher concentration of heavy metals in its composition.

AUTHOR CONTRIBUTIONS

F.D.B. and C.A. conceived and designed the experiments; F.D.B. performed the sample collection and processing, the determination of heavy metals. F.D.B. and C.A. contributed to statistical analysis and manuscript revision. T.G., E.V., O.C., M.A., C. G.G. and N.S contributes to data analysis and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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BEHAVIOR OF SOME GRAPEVINE CULTIVARS FROM MURFATLAR VINEYARD IN THE SPECIAL CLIMATIC CONDITIONS OF THE WINE YEAR 2019-2020

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ABSTRACT

The climate changes in Murfatlar vineyard in recent years consist inthe increase ofmonthly average temperatures, recorded in both cold and warm seasons, accompanied by an irregular distribution of rainfall during the year, which significantly influences the growth of grapevine, the quality and production of grapes.Six representative cultivars were studied, three for white wines: Chardonnay, Columna and Muscat Ottonel and three for red wines: Fetească Neagră, Mamaia and Cabernet Sauvignon. In the last two years (2019-2020) warm winters and very dry summers have led to the onset of budburst, almost simultaneously for all the studied cultivars, followed by a very weak growth and development of shoots (2020) and an acceleration development of phenophases, mainly berween veraison and ripening. Water stress during the vegetative period, a hygroscopicity below 60% between July and August and low vegetative apparatus influenced the growth ofthe berries, resulting small grapes, and a very low must yield in 2020.Concerning the quality of grape production, the studied cultivars achieved more sugar concentrations in berries up to 35.8 g/L (in 2019) and 36.0 g/L (in 2020), higher than the values obtained in normal years. In the conditions of a pronounced dry climate manifested in the two years of study, the productions were below the normal level, especialy in 2020, achieving much diminished productions for all cultivars, except the Mamaia cultivar. The Mamaia cultivar had a positive reaction, registering an increase of production, that exceeded the multiannual value by 20% in 2020 andby 10% in 2019, the concentration of sugars in the must being above the average value obtained in normal years, exceeding by 3% and 9%, respectively, the multiannual average. Statistical calculations were done using SPSS Statistics 17.0, using mainly the Duncan test for a degree of significance of 5%.

Keywords: climate change, phenology, cultivar adaptability, productivity and quality

INTRODUCTION

Climate changes in recent times had led to an increase in the average annual temperature, based mainly on average monthly values in winter and summer, a decrease in rainfall, much lower in summer, and an increase in the frequency of very drought years.
All these changes lead to a rapid development of vegetative phenophases (Cichi, 2006; Dejeu et al., 2008) leading to a shortening of the period of development and maturation of grapes and a low productivity in vineyards.

The choice of vine cultivars with increased adaptability to abiotic stress along with the improvement of cultivation technology is the most efficient way to ensure a constant and quality wine production.

MATERIALS AND METHODS

To describe the reaction of vine cultivars to climatic factors specific to the wine year and to the local area, between November 1, 2018 and October 30, 2020, observations were made on the succession of vegetative phenophases (noting the date when 50% of plants have physiologically reached that stage), using 3 grape cultivars for white wines and 3 for red wines, representative for the Murfatlar viticultural center: Columna, Muscat Ottonel, Chardonnay, respectively Mamaia, Fetească Neagră and Cabernet Sauvignon.
Research on the behavior of these cultivars was carried out in the plantations of the Research and Development Station for Viticulture and Oenology Murfatlar.
The studied cultivars were grafted on the same rootstock, the Oppenheim Selection 4 clone 4 and training system form adopted was the classic Guyot, with a fruit load of 34-38 buds per stem and a planting distance of 2.2/1.1 m. The plots cultivated with the Columna, Mamaia, and Cabernet Sauvignon cultivars are located on a land with E-W exposure and the plots cultivated with Chardonnay, Muscat Ottonel and Feteasca neagră have an N-S exposure, with slope of 2-3% and a calcareous chernozem soil with a clayey texture and a granular-porous glomerular structure with a humus content of 2.3%.
For monitoring the climatic elements, we utilised our own meteorological station (iMetos 3.3) located in the center of the vine plantation. Daily observations were made concerning the maximum and minimum temperatures, sunshine, relative air humidity and precipitation based on which the evolution of abiotic stress in the two wine years (November 2018 - October 2020) was determined compared to the multiannual average (1989-2018).
In order to characterize the heliothermal and hydric resources during this interval, a series of synthetic ecological indicators were used: the real heliothermal index (Branas et al., 1946); the hydrothermal coefficient (Seleaninov, 1936); the bioclimatic index of the vine (Constantinescu et al., 1936) and the oenoclimatic aptitude index (Teodorescu, 1987), the heliothermal index (IH) (Huglin,1978)and the cool night index (IF) (Tonietto et al., 2000).
In order to determine the drought affect on the vegetative growths, at the beginning of veraison, when the intense growth of the shoots ceased, the minimum, maximum and average length of the shoots was determined.The grape production was determined at harvest, establishing the average production per hectare.For the determination of the sugar content (g/L) the refractrometric method was used (Babeș, 2011) and for the total acidity (g /L H_2SO_4) the titrimetric method.

RESULTS AND DISCUSSIONS

During the rest period, the average monthly temperatures recorded registere an average thermal increased than average with 2.7˚ to 4.1 C.In the first part of the period of active vegetation, that includes the phenophases budburst and shoot growth, the average monthly temperature increased by 2.0-2.4˚C in the both studied years. The average temperatures in July, August and September have higher values by up to 6.6˚C (June 2019) being favorable for obtaining quality grape productions. (Figure 1).

The amount of precipitation recorded in the wine year 2018-2019 represent 60% of the multiannual average (311.4 mm) and in the wine year 2019-2020 represent66% of the multiannual average (340.5 mm). During the vegetative period, the amount of precipitation was 139.6 mm in the wine year 2018-2019 (106.1 mm less than the

multiannual average) and 161.7 mm in the wine year 2019-2020 (84 mm below the multiannual average) (Figure 2).

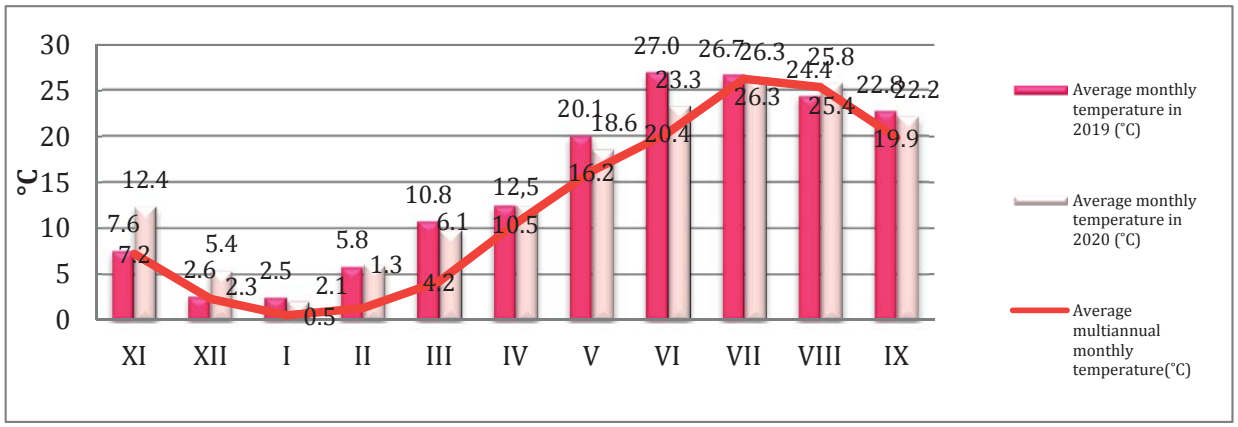

Figure 1. The evolution of the average monthly temperature period of grapvine, Murfatlar, 2019-2020

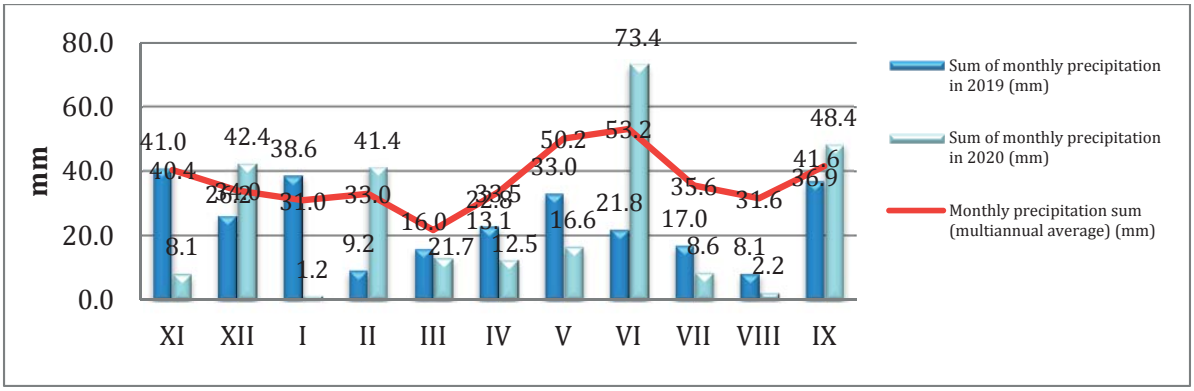

Figure 2. The sum of precipitation recorded, Murfatlar, 2019-2020

In 2018-2019 the relative humidity of the air during the vegetation period had normal values, however, in the wine year 2019-2020, in April, during the budburst phenophase, the atmospheric hygroscopicity was below 60% and during the berry growth and maturation it had values of 51-53%. (Figure 3).

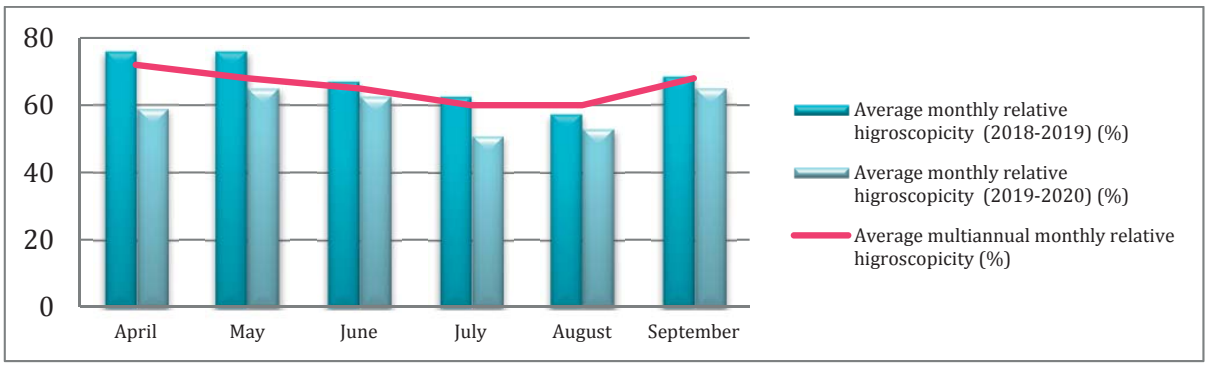

Figure 3. Montly relative air higroscopicity registered during the vegetative period of grapevine, Murfatlar, 2019-2020

The interval between November 2018 and October 2020 was characterized by the following ecoclimatic elements: the average annual temperature compared to the multiannual average (1989-2018) was 1.3˚C higher in 2019 and 1.6˚C in 2020. The global and active thermal balance had values with 520-565˚C higher than the multiannual average and the useful thermal balance in the wine year 2018-2019 was 2811,8˚C, and 2477,3˚C in the wine year 2019-2020, being with 506˚C, respectively 173,5˚C, more than the multiannual average value(Table 1).

The real heliothermal index (IHr) in Romania varies between 1.35 and 2.70. The average value of this index was 3.9. In the period 2019-2020 this index had increased values in the range of 4.0 - 4.5, which indicates rich heliothermal resources.

The hydrothermal coefficient (CH), in the Murfatlar viticultural centre has an average multiannual value of 0.8 and in the studied interval it had values of 0.4 indicating the need of irrigations.

The bioclimatic index of the vine (Ibcv), presents on the Romanian territory a pronounced variation, from the value 4.0 (in the vineyards of the north of the country), to 15.0 in the south.The value determined for this interval was 22 and 18, indicating years rich in heliothermal resources but very deficient in precipitation.

The oenoclimatic aptitude index (IAOe) presents a certain zonality in Romanian viticulture, having increasing values from the cooler climate (3700) to the warm climate (5200). In the Murfatlar vineyard this index has a multiannual average value of 5331.7 and in the studied years it was close to the multiannual average(5538.7 in 2019-2020 and 5847.7 in 2018-2019) that indicate favorable conditions for obtaining quality red wines.

The Huglin heliothermal index (IH) indicates the optimal conditions for obtaining quality grapes. In the wine year 2018-2019 the value of this index was 4583.7, higher than the multiannual value (3243.4) and registered a value very close to it in 2019-2020 (3138.8).

The cool night index has average value of 14.2 and in the years studied it had values of 14.4 - 14.5, indicating a climate with temperate nights.

Table 1. Values of the climate factors recorded between November 2018 and October 2020 at Murfatlar

Analyzed climatic elements	Average nov.1989 - oct.2018	Viticultural year	
		2018-2019	2019-2020
Global thermal sum, (∑t°g)	4982.4	5534.4	5515.4
Active thermal sum, (∑t°a)	4496.3	5061.8	5016.4
Useful thermal sum, (∑t°u)	2303.8	2811.8	2477.3
Average temperature in July, °C	26.3	26.7	26.3
Average temperature in August, °C	25.4	27.5	25.8
Average temperature in September, °C	19.9	22.8	21.7
Absolute minimum air temperature,°C	-14.5	-10.5	-13.9
Absolute minimum temperature at soil surface,°C	-15.6	-15.0	-13.7
Average annual temperature °C	13.5	14.8	15.1
Average monthly temperature in the rest months °C	3.1	5.8	7.2
Maximum air temperature,°C	37.3	39.0	39.0

Sum of annual precipitation, mm	514.5	311.4	340.5
Sum of precipitation during the rest period, mm	160.1	131.0	106.2
Sum of precipitation during the vegetation period, mm	317.4	180.4	161.7
Sum of insolation hours during the vegetation period, hours	1667.8	2125.5	1574.7
Average maximum temperatures in August,°C	31.2	33.8	30.4
Average temperatures in the I and II decades of July	23.4	26.3	22.1
Number of days with maximum temperatures > 30°C	56	98	63
Real Heliothermic index (IHr)	3.9	4.5	4.0
Hydrothermic coefficient (CH)	14.4	0.4	0.5
Bioclimatic index (Ibcv)	14.2	22.6	18.0
Enological aptitudie index (IAOe)	5321.7	5847.5	5538.7
Huglin heliothermic index (IH)	3243.4	4583.7	3138.8
Cool night index (IF)	14.2	14.4	14.5

In this climatic context, the vegetative phenophases had a much faster development in 2020 (Table 2).

Table 2. Date of completion of the main vegetative phenophases for the studied cultivars, Murfatlar, 2019-2020

CULTIVAR	**YEAR**	Phenophases					Days from budburst until maturation
		Budburst	Flowering	Veraison	Full maturity	Harvest date	
Cabernet Sauvignon	2019	30.04	08.06	17.08	23.09	30.09	147
	2020	26.04	05.06	18.08	18.09	24.09	146
Chardonnay	2019	18.04	06.06	07.08	09.09	17.09	145
	2020	16.04	03.06	04.08	28.08	02.09	135
Muscat Ottonel	2019	21.04	04.06	02.08	10.09	14.09	143
	2020	19.04	04.06	06.08	28.08	31.08	132
Fetească Neagră	2019	24.04	08.06	09.08	09.09	10.09	139
	2020	22.04	04.06	09.08	04.09	11.09	136
Columna	2019	23.04	09.06	14.08	12.09	17.09	143
	2020	21.04	04.06	12.08	10.09	16.09	143
Mamaia	2019	26.04	04.06	09.08	15.09	16.09	143
	2020	21.04	04.06	11.08	10.09	18.09	143

The studied cultivars had a comparable development of phenophases during the twoo studie years, being observed a shortening of the period between veraison and full maturity, mainly in the 2020.

At the beginning of veraison, when the intense growth of the shoots has stoped, the minimum, maximum and average length of the shoots was determined. For these, shoots from 5 trunks of each cultivar were measured and it was observed that in 2020 the shoot growth was 39% lower than in 2019. The lowest shoot growth was recorded in the Chardonnay cultivar (38.9 cm) (Table 3).

Table 3. Data concerningvine shoot lengths at the beginning of veraison, Murfatlar, 2019, 2020

No.	Cultivar	Shoot length (cm)					
		Wine year 2018-2019			Wine year 2019-2020		
		Minimum (cm)	Maximum (cm)	Average shooth length (cm)	Minimum (cm)	Maximum (cm)	Average shooth length (cm)
1	Chardonnay	87.2	154.1	136.2	18.0	51.4	38.9
2	Columna	96.4	232.2	176.4	39.5	213.1	62.3
3	Muscat Ottonel	41.4	1683	121.2	27.0	131.5	56.1
4	Fetească Neagră	103.2	2371	189.4	26.5	170.2	95.3
5	Mamaia	104.5	218.6	182.4	13.0	105.5	54.7
6	Cabernet Sauvignon	48.1	176.5	139.2	32.0	88.0	45.7

The weight of 100 berries (g), together with the sugar content (g/L) and the total acidity (g/L H_2SO_4) are the main analytical characteristics that offer a complete characterization of the grapes at harvest (Table 4).

Comparing the elements of productivity and quality that characterize the vine cultivars in the studied wine years (under conditions of an excessive water stress during the vegetative period) with the average of 2014-2018, it can be seen that in the wine year 2019 the productions were close to the average multiannual value for Columna (5074 kg compared to 5070 kg), Cabernet Sauvignon (4884 kg compared to 5160 kg), Chardonnay (5096 kg compared to 5606 kg).Yields were above the annual average for Muscat Ottonel (5013 kg compared to 3895 multiannual average) and Mamaia (5781 kg compared to 4803 kg multiannual average), and below the multiannual average for the Fetească Neagră cultivar (1694 kg lower than the multiannual average).

In 2020, the water stress induced by the lack of precipitation during both the rest and the vegetative period (which led to an irregular budburst and development of the vegetative apparatus), determined productions per hectare below the average value of 2019. The lowest yields were recorded for Chardonnay and Cabernet Sauvignon cultivars (minus 532 kg/ha and minus 1188 kg/ha), which had a vegetative apparatus based on shoots with low growth (average length of shoots reached 38.9 cm in Chardonnay and 45.7 cm in Cabernet Sauvignon) develeopedirregular along the cane length. For Columna, Muscat Ottonel and Fetească Neagră cultivars, the production was 50% below the average of 2014-2018, and for the Mamaia cultivar the average production per hectare was higher than the multiannual average (4803 kg/ha).The average weight of 100 berries for all cultivars was below the multiannual average in 2020, but in 2019 only for the Columna and Muscat Ottonel cultivars was lower and higher for the other cultivars.

Table 4. Data concerning the productivity and quality of the harvest for the studied cultivars, Murfatlar, 2019-2020

Cultivar	Year	Average yield		Weight of 100 berries (g)	Physico-chemical characteristics of the must	
		kg/ha	kg/trunk		Sugars (g/L)	Total acidity (g/LH_2SO_4)
Chardonnay	2014-2018 Average	5606±50 (a)	1,356±0,25 (a)	112±5 (b)	221,9±5,7 (b)	5,21±0,72 (a)
	Wine year 2019	5096±37 (b)	1,23±0,20 (a)	141±7 (a)	223,5±6,2 (b)	4,5±0,48 (ab)
	Wine year 2020					
Columna	2014-2018 Average	532±10 (c)	0,128±0,03 (b)	98±3 (c)	246±4,5 (a)	3,62±0,31 (b)
	Wine year 2019	5070±45 (b)	1,338±0,33 (a)	192±8 (a)	179,9±3,7 (a)	4,42±0,52 (a)
	Wine year 2020					
Muscat Ottonel	2014-2018 Average	5174±270 (a)	1,365±0,37 (a)	186±7 (a)	179,9±3,1 (a)	3,5±0,47 (a)
	Wine year 2019	2640±30 (c)	0,696±0,08 (b)	158±5 (b)	181,1±2,9 (a)	3,75±0,35 (a)
	Wine year 2020					
Fetească Neagră	2014-2018 Average	3895±36 (b)	0,974±0,05 (a)	169±4 (a)	217,2±4,5 (a)	4,13±0,30 (a)
	Wine year 2019	5013±49 (a)	1,213±0,21 (a)	150±3 (b)	219±3,6 (a)	3±0,25 (b)
	Wine year 2020					
Mamaia	2014-2018 Average	2836±30 (c)	0,684±0,03 (b)	115±3 (c)	220,2±4,0 (a)	3,48±0,37 (b)
	Wine year 2019	6382±52 (a)	1,679±0,30 (a)	126±7 (b)	220,6±5,0 (b)	4,24±0,20 (a)
	Wine year 2020					
Cabernet Sauvignon	2014-2018 Average	4688±40 (b)	1,134±0,15 (b)	142±9 (a)	256,4±5,8 (a)	4,2±0,27 (a)
	Wine year 2019	2200± 31 (c)	0,532±0,03 (c)	125±5 (b)	256,6±5,3 (a)	3,16±0,22 (b)
	Wine year 2020					

Mean values ± standard deviation (n=3). The letters denote the significance $p<0.05$ of the differences among data. Any two values followed by at least one common letter do not differ significantly.

The quality of production in 2020 was superior to the average of 2014-2018 for all the studied cultivars. The highest concentration of sugars was recorded for the cultivars Fetească Neagră (256.6 g/l) and Chardonnay (246.9 g/L).

Compared to the multiannual average, the concentration of sugars (g/L) accumulated in 2019 was higher for Mamaia (206.6 g/L), Chardonnay (223.5 g/L), Fetească Neagră (highest sugar concentration - 256.4 g/L) and Cabernet Sauvignon (210.8 g/L).

The total acidity of grapeshad values between 3.00-5.0 g/L H_2SO_4, below the multiannual average, generally low, due to the specificity of the Murfatlar viticultural area.Water stress during the vegetative period, hygroscopicity below 60% in July and August and a reduced vegetative apparatus directly influenced the growth and development

of the berries, obtaining a very low must yield in 2020 with values in the range of 50.0 - 61.2% (on average 19.0% lower than the average of 2014-2018 and 15% below the average of 2019). In 2019, the must yield was 66.7 - 69.8% (on average 4% less than the averageof 2014 - 2018) (Figure 9).In 2020, most cultivars had a must yield of 50-51%, less Columna and Mamaia cultivars, which had a yield of 60.0% and 61.2%, respectively.

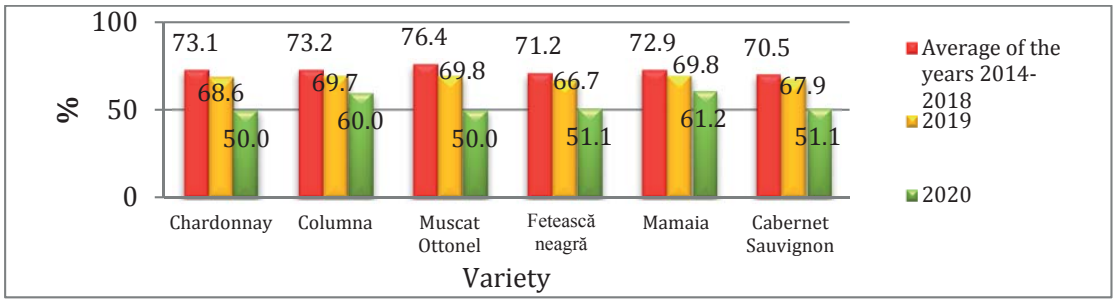

Figure 9. Grapes yield must for the studied cultivars, Murfatlar 2019-2020

CONCLUSIONS

- The special climatic conditions (drought and high temperatures) manifested during the period of rest and active vegetation of the vine influenced in a strong manner the vegetative phenophases, the growth and development of shoots, productivity and quality of production.
- The cultivars studied in 2020 registered an irregular start in vegetation, influencing the growth and development of the vegetative apparatus, but also the fertility and productivity of the trunks.
- The small amount of precipitation and the relative humidity of the air, with values below 60% (51-53%), directly influenced the growth and development of the berries, obtaining small grapes, not specific to the studied cultivars and a must yield below the average multiannual value, by 4% in 2019 and 19.0%in 2020.
- From the point of view of the quality of grape production, the studied cultivars achieved concentrations in sugars above the average of 2014-2018. The highest sugar concentrations were accumulated in Fetească Neagră and Chardonnay (256.6 g/l and 246.9 g/l respectively) in 2020.
- In the conditions of a pronounced dry climate, manifested in the two years of study, only Mamaia cultivar registered a higher production (5289 kg/ha compared to 4803 kg/ha - average of 2014 -2018), a must yield of 61,2% (in 2020), 69.8% (in 2019) and a sugar concentration of 206.6 g/L (in 2019), respectively of 204,5 g/L (in 2020) with an acidity of 3,50 and 3,68 g/L H_2SO_4.

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OENOLOGICAL CHARACTERIZATION OF SOME YEAST STRAINS ISOLATED FROM THE IAŞI VINEYARD ROMANIA

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ABSTRACT

This study investigated the oenological potential of indigenous*Saccharomyces* and non-*Saccharomyces* yeasts isolated from different stages of the natural must fermentation process. Screening of extracellular enzymatic activities was performed on agarized media in which the following substrates were added: arbutin, cellobiose, Tween 80, tributyrin, casein and citrus pectin, to highlight the activity of enzymes: ß-glucosidase, esterase, lipase, protease and pectinase. Among the 30 *Saccharomyces cerevisae* strains tested, 37% showed very low β-glucosidase activity, 100% showed esterase activity, 40% lipase activity, 90% protease activity and 53% pectinase activity.The non-*Saccharomyces* strain tested showed β-glucosidase, esteraseand protease activity. Tolerance to ethanol was assessed in YPD medium with ethanol concentrations of 5, 10 and 15% (v/v) by yeast culture development index.At 15% ethanol, development of all tested strains were inhibited. In the case of SO_2 tolerance, decrease in strains development was inversely correlated with the increase in potassium metabisulphite concentration, up to 200 mg/L. Only four *Saccharomyces* strains showed specific oenological characteristics and were selected to be tested in mixed and/or sequential cultures to obtain wines with improved sensory features.

Keywords*:* wineyeasts, *Saccharomyces*, non-*Saccharomyces*, extracellularenzymes, ethanol tolerance.

INTRODUCTION

The use in winemaking of the starter cultures of *Saccharomyces cerevisiae* had the advantage of a controlled alcoholic fermentation process, but also the disadvantage of obtaining wines with deficiencies in terms of aromatic profile. Flavours are produced during the complex biochemical process of fermentation by extracellular enzymes secreted by yeasts. In the case of spontaneous fermentation, the range of extracellular enzymes is much larger and varied due to the participation of many yeast species both *Saccharomyces* and non-*Saccharomyces*. This explains why the wines obtained in natural alcoholic fermentation have a much more complex aromatic profile, compared to the wines obtained in monoculture with *Saccharomyces cerevisiae* starter yeast. The current practice of inhibiting the *non-Saccharomyces* yeast population which predominates at the beginning of alcoholic fermentation, by inoculation of *Saccharomyces* starter cultures, need to be reconsidered, due to the fact that research conducted by different authors revealed that some of these yeast strains showed superior oenological characteristics, with positive effects on the aromatic profile of wines.Soden et al. (2000) showed that a positive impact on the sensory

characteristics of wine is ensured by the involvement of non-*Saccharomyces* yeasts in the alcoholic fermentation, due to their potential to secrete extracellular enzymes (β-glucosidases, esterases and lipases). Thus, in the last years, the research focused on the study of extracellular enzymes of *Saccharomyces* and non-*Saccharomyces* yeasts, finding that the presence or absence of the enzymes influences the wine aromas (Strauss et al., 2001; Buzzini and Martini, 2002; Rodriguez et al., 2004; Ciani et al., 2006; Mendoza et al., 2007; Gaensly et al., 2015; López et al., 2015). The surveys carried out in recent years allowed the selection of new strains of wine yeast both *Saccharomyces*, but especially non-*Saccharomyces*, in order to obtain and use them in mixed cultures, to improve the flavours complex of thewine and toenhance regional identity of wines.Thus, the research opened a way to increase the sensory attributes of wines, in new winemaking technologies that involvesthe management of alcoholic fermentation in similar conditions to the natural process, but initiated with both *Saccharomyces* and non-*Saccharomyces*yeasts starter cultures. In this context, oenological characterization of indigenous *Saccharomyces* and non-*Saccharomyces* yeasts was initiated in order to identify and select performant strains in terms of extracellular enzyme activity, which by biochemical transformations of must compounds ensure the variety and the complexity of the desired sensory characteristics of wines. Also, by testing them in mixed and/or sequential cultures, important steps will be taken in increasing the quality and tipicity of wines.

MATERIALS AND METHODS

The evaluation of the oenological characteristics of the indigenous yeast strains was focused on the determination of the extracellular enzymatic potential, the tolerance to ethanol and SO_2. In this context, 30 *Saccharomyces cerevisiae* strains and one non-*Saccharomyces* strain, respectively *Torulospora delbrueckii*, were studied.

Screening for extracellular activities of *Saccharomyces* and non-*Saccharomyces* yeasts was performed on agar YPD medium(containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 20 g/L agar), inoculated with suspensions of 10^6 cells/mL from yeast cultures reactivated 24 hours at 25°C.

β-glucosidase activitywas determined on agar media containing 6.7 g/L Yeast Nitrogen Base - YNB (Sigma-Aldrich, Germany), 5 g/L arbutin (Fluka, Switzerland), 20 g agar(VWR, Belgium),pH 5.5. After autoclaving were added to 100 mL medium: 2 mL of 1% ferric ammonium citrate solution (Rossi et al., 1994). Yeasts that hydrolyzed the substrate produce β-glucosidase and a brown halo appears around the colonies. The strains which developed on cellobiose agar (10 g/L cellobiose, pH5.5) and hydrolysed at least one other substrate (either arbutin or esculin) were positive toβ-glucosidase production (Gaensly et al., 2015). Esterase activitywas tested on medium with the following composition: 10 g/L peptone, 5 g/L NaCl, 0.1 g/L $CaCl_2 \cdot 2\ H_2O$, 10 g/L Tween 80, 20 g/L agar, pH 6.8 (Slifkin, 2000). Yeasts with esterase activity hydrolysed the substrate and an opaquehalo appeared around the colonies. The assay of the protease activity was performed on an agar medium with the following composition: 3 g/L yeast extract (Scharlau,Spain), 3 g/L malt extract (Merck, Germany), 5 g/L peptone, 10 g/L glucose(VWR, Belgium), 5 g/L NaCl(Chemical Company, Romania) and15 g agar (Comitini et al., 2011).Separately, was prepared a solution containing an equal volume of pasteurized skimmed milk (0.1% fat) and sterile distilled water.After sterilising the medium, both solutionswere homogenised and brought to 45°C.The medium was distributed in Petri dishes and then was inoculated with the yeast suspension. Proteolytic yeasts hydrolyzemilk casein and a clear halo appears around the inoculum. Lipase activitywas tested on tributyrin agar medium with the following composition: 5 g/L peptone, 3 g/L yeast extract, 10 g/L tributyrin(Fluka, Switzerland), 15

g/L agar, pH 6.0 (Buzzini and Martini, 2002). After incubating the plates for 48 hours at 28°C, the detection of lipase activity was performed by UV irradiation at 350 nm. In the case of yeasts with lipolytic activity, a fluorescent halo was observed. Highlighting the pectinase (polygalacturonase) activity was performed according to McKay (1990) with some modifications. The medium contains: 10 g/L of citrus pectin (Sigma-Aldrich, Germany), 6.8 g/L KH_2PO_4, 6.7 g/L YNB (Sigma-Aldrich, Germany), 6.7 g D-glucose (VWR, Belgium) and20 g/L agar.Yeast suspensions were inoculated on the surface of the agar medium. The plates were incubated for 3-5 days at 30°C and then flooded with a 10 g/L hexadecyltrimethy-lammonium bromide solution (Sigma-Aldrich, Germany). The development of a clear halo around the colonies indicates the pectinase activity.

SO_2 tolerance was performed on sterilized YPD medium with potassium metabisulphite in concentrations of 50, 100, 150 and 200 mg/L. Control was represented by the same medium without $K_2S_2O_5$addition. From the reactivated yeast cultures on YPD medium, were obtained suspensions of 10^4cells/mL. After inoculation and incubation at 26°C for 48 hours, the development of yeasts (positive or negative) was examined compared to the control medium. Resistance degree of yeast to sulphur dioxide is reported as the maximum dose at which the yeast exhibits a significant growth. Evaluation of ethanol tolerance of indigenous yeast strains was performed on 5 mL of YPD medium with ethanol concentrations of 5, 10 and 15% (v/v).Each ethanol concentration was inoculated with 100 µL of a 24-hour culture. Yeast strain development was monitored for 72 hours by measuring the optical density of the cultures at 600 nm, using YPD medium as the blank sample (Analytik Jena Specord 200 plus UV-vis spectrophotometer).The control sample consisted in 5 mL YPD medium without ethanol inoculated with 100 µL of each yeast culture. Tolerance to ethanol was determined based on the growth index (GI), calculated according to the following formula proposed by Bevilacquaet al. (2012): GI% = (Abs sample/Abs control) × 100. The degree of inhibition was established as follows: GI<25% = very high degree of inhibition; 25 <GI<75% = partial inhibition; IG>75% increase similar to the control sample (without ethanol).The values of the growth index represent the average of three determinations, standard deviation being calculated (±).

RESULTS AND DISCUSSIONS

The purpose of the research was to select indigenous yeast strains with superior oenological characteristics for use in the process of alcoholic fermentation in mixed and/or sequential cultures, in order to improve the aromatic profile of wines and to enhance their regional identity. Yeast strains were isolated in different stages of natural (spontaneous) alcoholic fermentation and are stored in the collection of microorganisms of Research and Development Station for Viticulture and Oenology Iași, Romania, on YPD medium, at 6°C.

The screening of extracellular enzyme activities was performed on agarized media in which specific substrates were added: arbutin, cellobiose, Tween 80, tributyrin, casein and citrus pectin, to highlight the enzymes β-glucosidase, esterase, lipase, protease and pectinase.Depending on the number of extracellular enzymes, 5 groups of tested yeasts were distinguished: three strains with five extracellular enzymes (group I); six strains with four extracellular enzymes (group II); 13 strains with three extracellular enzymes (group III); six strains with two extracellular enzymes (group IV) and one strain with a single enzyme (group V).The data presented in table 1 highlight the following aspects: all yeast strains tested show different extracellular enzymatic activities; β-glucosidase activity was not detected on the arbut in substrate, instead, on the cellobiose substrate 11 strains were positive; the esterase activity was positive in all strains, and the enzymatic activities of lipase, protease and pectinase were highlighted in 12 strains, 27 strains, respectively 16

strains. Another aspect regarding the screening of *Saccharomyces cerevisiae* yeasts emerged from the assessment of the intensity of extracellular enzyme activities (Figures 1 - 5).

Table 1. Extracellular enzymatic activity of the tested yeast strains

No.	Yeast strain (code)	Extracellular enzymatic activity					
		β-glucosidase		Esterase	Lipase	Protease	Pectinase
		arbutin	cellobiose	Tween 80	tributyrin	casein	pectin
Saccharomyces cerevisiae							
1	1	-	-	+	-	-	-
2	3	-	+	++	+	+	++
3	4-1	-	-	+	+	++	-
4	4-1-11	-	+	++	+	+++	-
5	4-3	-	+	++	+	+	++
6	4-5	-	-	+	-	+	+++
7	4-6	-	+	+	+	+	++++
8	4-7	-	-	+	-	+	++++
9	4-8	-	-	+	-	+	+++
10	4-10	-	+	++	+	-	+++
11	4-12	-	-	+	-	+	+++
12	4-13	-	-	+	-	+++	+
13	4-14	-	-	++	-	+	-
14	4-15	-	+	++	-	+	+++
15	4-16	-	-	++	+	++	-
16	4-17	-	+	+	-	++	-
17	4-17-341	-	-	+	+	+++	-
18	4-19	-	-	++	-	++	-
19	4-20	-	-	+	-	++	-
20	4-21	-	-	+	+	+	-
21	5-1	-	+	+	-	+	+
22	5-2	-	-	+	-	+	+
23	6-1	-	+	+	-	+	+++
24	6-2	-	+	+	+	+	+
25	6-3	-	+	++	+	+++	-
26	6-6	-	-	++	-	+++	-
27	7-2	-	-	++	-	+	++
28	7-3	-	-	++	-	-	++
29	8-1	-	-	++	-	+++	-
30	8-2	-	-	++	+	+++	-
Torulospora delbrueckii							
1	10	+	+	-	+++	-	+
Note:–no activity; + very low activity; ++ low activity; +++ high activity; ++++ very high activity.							

The importance of β-glucosidase in winemaking is determined by its potential to release flavor compounds. In wine must, some secondary metabolites are free or bound, the latter can be hydrolysed under the action of yeast enzymes. The enzymes involved in the hydrolysis of flavor precursors are glycosidases, respectively β-glycosidase which releases monoterpenes from the glycosylated form (Maicas and Mateo, 2005). Volatile compounds released from glycoside complexes play an important role in obtaining varietal aromas. Although some strains of *Saccharomyces cerevisiae* produce β-glucosidase, several studies have shown that some non-*Saccharomyces* yeasts have higher activity.The intensity of β-glucosidase activitywas identified in 11 strains of the 30 *Saccharomyces cerevisiae* yeasts tested, with a very low intensity (+) on the cellobiose substrate (figure 1).The difference was clear between the Petri dishes inoculated with the negative strains compared to the positive ones, for example strain 7-2 versus strain 4-10 (figure 1).The results obtained are in agreement with previous research. Rosi et al. (1994), Rodríguez et al. (2004) and Comitini et al. (2011), using arbutin as a substrate, among a high number of strains found β-glucosidase activity only for a single *Saccharomyces cerevisiae* strain.

The esterase activity was highlighted on agar medium added with Tween 80.Hydrolysis of the substrate by esterases led to the development of a visible opaque halo by precipitation of calcium ions. Among the *Saccharomyces cerevisiae* yeast strains, 16 showed very low activity (+)(e.g. strain 4-15) and 14 showed low activity (++) (e.g. strain 4-1-11) (figure 2).

Although lipase activity was found in yeasts, there are few references to its intensity in *Saccharomyces cerevisiae* strains. From the total of 30 strains tested, only in the case of 12 strains was determined a very low activity (+). The intensity of enzyme activity was assessed both by the presence of an opaque halo and by the appreciation of yeast development on the surface of agar media.The lack of activity was proven by the absence of inoculum development (figure 3).

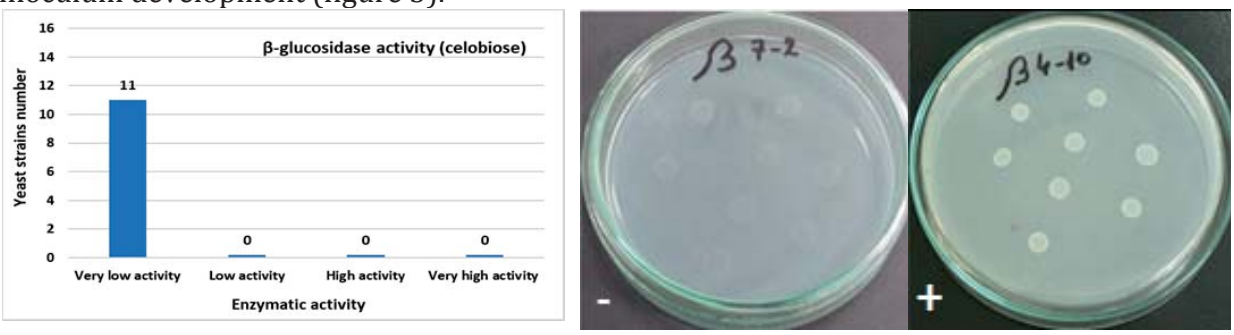

Figure 1. Number of yeast strains that showed β-glucosidase activity and the intensity of growth

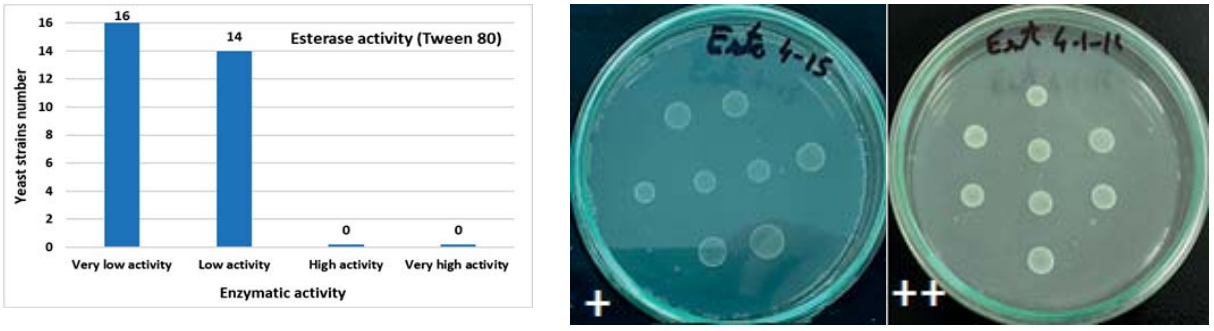

Figure 2. Number of yeast strains that showed esterase activity and intensity of the enzymatic activity

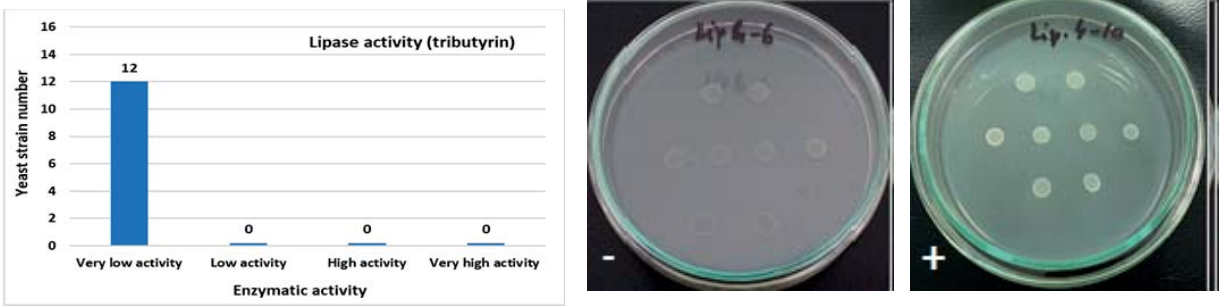

Figure 3. Number of yeast strains that showed lipase activity and intensity of the enzymatic activity

Lipase hydrolyses the long chains of triglycerides in the lipids present in the must and those resulting from the autolysis of yeasts. The enzymatic activity of lipase favours the extraction of colour from the skin of grapes and also the release of volatile compounds, such as ethyl esters and ethyl acetates that influence the aroma of wines.

Proteolytic activity has been studied for the stabilization and prevention of protein haze, where proteins are hydrolysed into amino acids and peptides. Protein haze is a problem in many white wines and the use of bentonite to stabilize it has disadvantages, both by losing wine volume and sensory properties (Maicas and Mateo, 2005). Information on the proteolytic activity of *Saccharomyces cerevisiae* yeasts is very scarce, but numerous in the case of *non-Saccharomyces*yeasts. The intensity of protease activity of the tested *Saccharomyces cerevisiae* strains was different on casein agarized medium (figure 4). Thus, 15 strains showed very low proteolytic activity (+), with a clear halo of 1 mm (strain 5-1), 5 strains showed low activity (++), with a clear halo of 3 mm and 7 strains with intense activity (+++) with a clear halo of 4-5 mm (strain 4-13).

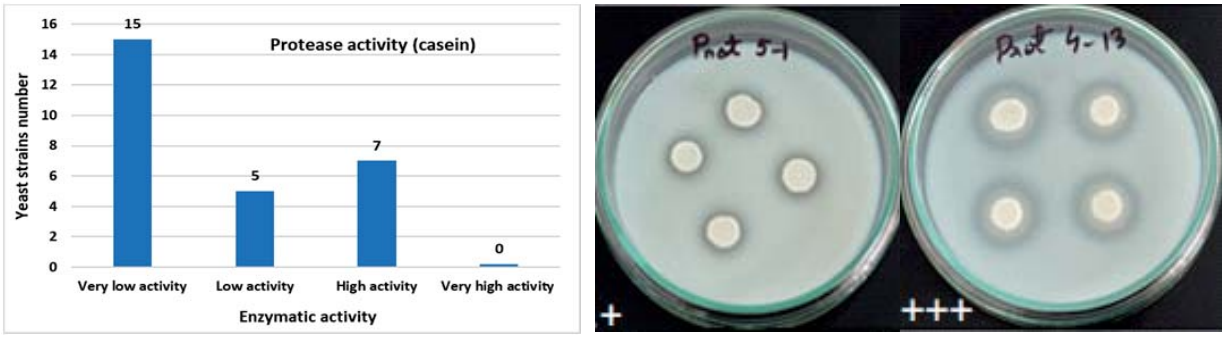

Figure 4. Number of yeast strains that showed protease activity and intensity of enzymatic activity

Among the pectinase enzymes group, this study determined the extracellular poly-galacturonase activity. Tested yeast strains showed variable intensity of polygalacturonase activity. The enzymatic intensity on an agarized medium was highlighted by a clear halo with different sizes from 1 to 5 mm.Depending on this parameter, the 16 positive strains were grouped as follows: 4 strains with very low activity (+), with a clear halo of 1 mm (strain 5.1), 4 strains with low activity (++), with a clear halo of 2 mm, 6 strains with intense activity (+++), with a clear halo of 4 mm and 2 strains with very intense activity (++++), with a halo of 5 mm (strain 4-7) (figure 5).

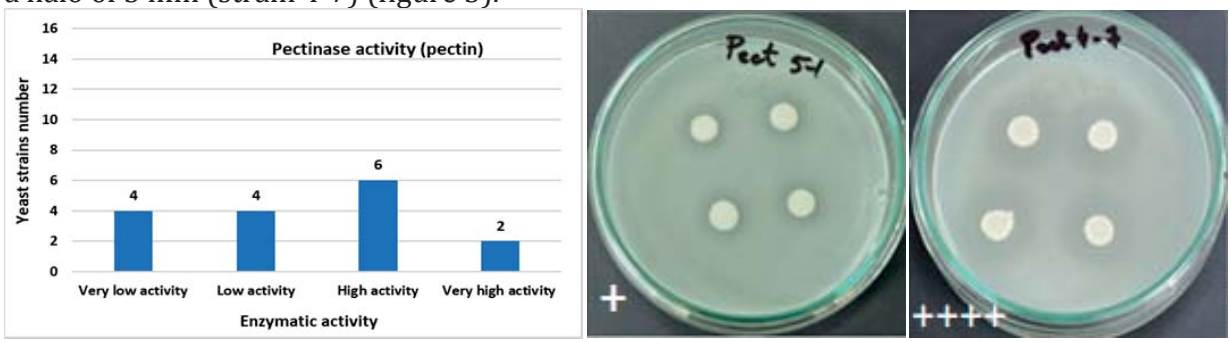

Figure 5. Number of yeast strains withpectinase activity and intensity of the enzymatic activity

Among extracellular enzymes, pectinases play an important role in oenology. These enzymes, mainly the depolymerizing ones, have a substrate specificity to cleave the oxide bonds 1-4 from the ends of the polygacturonic chain (exoenzymes) or from its middle (endoenzymes).By the action of these enzymes, the viscosity of the must decreases. The main wine yeast *Saccharomyces cerevisiae* has not been appreciated as a major producer of pectinase extracellular enzymes, although several strains have been shown to produce polygalacturonases (McKay, 1990).

Due to the small number of recent references on the extracellular enzymatic activity of *Saccharomyces cerevisiae* wine yeasts, the study has a double importance, in the way that the enzymatic potential of the wine yeasts was studied for the first time in Romania and, also, the study contributes to updating and completing data on this issue at international level.

In current study was also assessed the intensity of the extracellular enzymes activity foranon-*Saccharomyces* strain, *Torulospora delbrueckii*.The non-*Saccharomyces* strain showed low β-glucosidase and pectinase activity (+),no esterase orprotease activity, but the highest lipase activity (Table 1).Buzzini and Martini (2002) tested five*Torulospora delbrueckii*strains, of which only one strain showed esterase and lipase activity. Also, Rodriguez et al. (2004) tested four strains on arbutin medium, all strains being β-glucosidase negative. Comitini et al.(2011) tested nine *Torulospora delbrueckii* strains of which only two showed β-glucosidase activity and five strains showed esterase activity.

From a total of 30 *Saccharomyces* yeast strains whose enzymatic potential was tested, 14 yeast strains were selected from the groups with 3, 4 and 5 secreted extracellular enzymes, in order to test the tolerance to ethanol and SO_2.
Yeast tolerance to ethanol was assessed based on the values of the growth index (GI), the results obtained being presented in Table 2.

Table 2. Tolerance to ethanol and SO_2 of the tested *Saccharomyces cerevisiae* yeast strains

Nr.	Strain code	Growth index (GI %)			SO_2, concentration				
		5% ethanol	10% ethanol	15% ethanol	Control	50 mg/L $K_2S_2O_5$	100 mg/L $K_2S_2O_5$	150 mg/L $K_2S_2O_5$	200 mg/L $K_2S_2O_5$
1	3	92.0±1.2	77.9±0.2	0.0	++++	+++	+++	++	-
2	4-1-11	89.8±0.8	85.3±1.0	0.0	++++	+++	+++	++	-
3	4-3	87.9±1.3	83.3±0.5	0.0	++++	+++	+++	++	-
4	4-5	90.0±1.1	82.0±1.8	0.0	++++	+++	+++	+	+
5	4-6	86.9±1.4	86.0±1.0	0.0	++++	+++	+++	+	-
6	4-7	85.0±0.6	84.0±1.1	0.0	++++	+++	++	++	-
7	4-8	90.0±1.0	86.0±1.4	0.0	++++	+++	+++	+	-
8	4-10	86.7±1.0	85.6±1.4	0.0	++++	+++	+++	+	-
9	4-12	89.1±1.2	79.4±0.9	0.0	++++	+++	++	++	+
10	4-15	93.0±0.8	76.0±1.0	0.0	++++	+++	+++	++	+
11	5-2	72.0±1.6	48.9±1.2	0.0	++++	+++	+++	+	-
12	6-1	43.6±0.4	25.0±1.0	0.0	++++	+++	+++	+	-
13	6-3	91.9±1.1	75.3±1.0	0.0	++++	+++	+++	++	-
14	7-2	68.0±1.0	46.1±0.6	0.0	++++	+++	++	+	-

Note: –no growth; + very weak growth; ++ weak growth; +++ intense growth; ++++ very intense growth.

According to the data presented in Table 2, it was found that at 5% ethanol (v/v), GI% values were < 75% for three strains, which corresponds to a partial inhibition (5.2, 6.1, 7.2). 11 strains showed GI values ≥75%,considered as similar in development to the control sample. At 10% (v/v) ethanol concentrations, one strain showed a high degree of inhibition (6.1) with GI of 25%, 2 strains a degree of partial inhibition (5.2, 7.2), respectively GI values <75% and 11 strains with GI values ≥75% indicating a development similar to that of the ethanol-free medium (control). At concentration of 15% ethanol (v/v), no tested strain has developed on the culture medium.The assessment of SO_2 tolerance was made according to the ability of the tested strains to develop at the concentrations of potassium metabisulphite used.The results obtained are presented in Table 2.

On the control medium (SO_2-free)all strains have developed very intensely (++++). At concentrations of 50 mg/L of potassium metabisulphite ($K_2S_2O_5$), no differences in culture development were observed between the tested strains, but, compared to the control, all strains showed a slightly attenuated development. Differences were reported between strains starting at concentration of 100 mg/L $K_2S_2O_5$, thus, 11 yeast strains had an intense development (+++) and 3 strains a low development (++).

With the increase of the concentration to 150 mg/L $K_2S_2O_5$, among the 14 *Saccharomyces*strains, nine were appreciated as having a low development (++) and five strains showing a very low development (+). At 200 mg/L $K_2S_2O_5$the inhibition of strain development was very high, 11 strains did not develop, only three strains showing a very low development.

CONCLUSIONS

In the screening of the extracellular enzymatic activity, the obtained results led to the differentiation of the *Saccharomyces cerevisiae* yeast strains according to the complexity of

the enzymatic potential represented by the number of secreted enzymes.Thus, the strains that produced at least four enzymes were considered important for the proposed purpose. The intensity of the enzymes activity was very different, varying depending on the tested yeast strain. Very low activity was found forβ-glucosidase enzyme in 11 strains and for lipase in 12 strains. In the case of esterase, 16 yeast strains showed very low activity and 14 strains low activity. In contrast, seven *Saccharomyces cerevisiae* strains presented intense protease activity. Tested yeast strains showed high tolerance to ethanol concentrations up to 10% (v/v), confirmed by high values of the growth index (> 75%). In the case of SO_2 tolerance, the decrease of strains development was correlated with the increase of the potassium metabisulphite concentration. Among the tested yeastsonly four*Saccharomyces* strains, showingspecific oenological characteristics were selected to be tested in mixed and/or sequential cultures to obtain wines with improved sensory features.

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INFLUENCE OF CLIMATE FACTORS ON YIELD AND QUALITY OF SOME VINE CULTIVARS FROM THE ȘTEFĂNEȘTI CENTER

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Abstract

The influence of climatic factors on grape yield, fertility indicators of grapes (absolute coefficient of fertility, relative coefficient of fertility, absolute productivity indices, relative productivity indices), but also sugar and total acidity content in three cultivars of 'Fetească' ('Fetească Albă', 'Fetească Neagră' and 'Fetească Regală', respectively) was evaluated during 2016-2020 2016 period at the experimental field of National Research and Development Institute for Biotechnology in Horticulture Ștefănești. The highest grape yield were recorded in 2018 to all three Cultivars analysed (20,33 t/ha 'Fetească Albă', 19,44 t/ha 'Fetească Neagră' and 19t/ha 'Fetească Regală', respectively), while the lowest values were measured in 2016 at all cultivars. Highest sugar content was measured in 2019 and 2020, and lowest in 2017, 2016, respectively. The highest acid content was recorded in 2017 and 2016 (4,64 g/l, 4,65 g/l, respectively) as a result of heavy rainfall during the growing season, especially in June and September. Differences between the studied parameters were statistically significant. The results of the five years showed that the yield and quality of grapes were in direct relation with the weather conditions in certain years of experiments.

Keywords: Feteasca cultivars, climatic factors, yield, quality of grapes, coefficient of fertility

INTRODUCTION

Romania's viticulture is recognized for vineyards and viticultural centres, known notonly for their fine wines, but also for the special quality of table grapes. The complexityof Romanian geographic landscape creates favourable ecoclimates for the cultivation of table grapes cultivars, known for being more demanding than heliothermic and hydricregimes. An increasing temperature was reported in Europe by many scientists, e.g. Guedon and Legave (2008) by about 1.1-1.3°C in France, Blanke and Kunz (2017) by 0.6°C and Waldauand Chmielewski (2018) by 1.9°C, the last two in various regions of Germany. Due to climate change with warmer winters and earlier springs, temperate fruit crop adaptation in many places will be at risk in the future (Wenden et al., 2017). In Romania, data on climate for the investigated region were first reported about two decades ago (Paltineanu et al., 2000), and the trend of warming has been noted by Paltineanu et al. (2012). More recently, Busuioc et al. (2015) reportedan increasing trend in air temperature during the 1962-2010 period in Romania, while Chitu et al. (2015), Florea et. al (2020) emphasized the increased variability of seasonal and annual extreme temperature trends of the latest three decades in the study

region. Moreover, the current climate warming favours this direction of production. Statistical data from 2018 show that the total area cultivated with vine for wine in Romania was 171.2 ha in 2016, 170.3 in 2017 and 171.1 ha in 2018 and the total wine production was 33030. 7 thousand hl in 2016, 4264.1 in 2017 and, respectively 5088.1 in 2018. (Ministry of Agriculture and Rural Development, Data source: point 1.2 - INS date - Online tempo, point 1.3 - MADR operative date, point 4). 'Feteasca Albă'cultivar is cultivated in 2018 on an area of 9,298 ha, 'Feteasca Regala' 14,010 ha and only 2950 ha are cultivated with 'Feteasca Neagră' (Ministry of Agriculture and Rural Development, Data Source– National Institute of Statistics). The total area under table grape cultivars is 8.993 ha and the total production is about 124.400 tones of grapes for fresh consumption (National Office of Vine and Wine Products –ONVPV, 2017). Numerous studies worldwide predict that extreme future weather conditions will be more frequent, and negative impact on agricultural productionwill be more noticeable. However, it should always be considered that in addition to agro ecological conditions (relief, exposure, temperature sums, radiation, physical and mineral properties of soil), agrobiological, economical and technological properties of grape vines are significantly affected by the level of applied ampelotechnics, the type of rootstock, growth form, the pruning technique and many others (Brighenti et al., 2010). All listed above impose a constant need for research ofimpact of changing climate on agro-technological and economical characteristics of the dominantly cultivated cultivars of vine in south of Romania, and therefore the cultivars'Feteasca'as well, which was one of the leading vine cultivars for the production of high quality wines. The aim of this study is to evaluate the impact of major climatic parameters on the yield and quality of grape of three cultivars of 'Feteasca', especially during the vegetation season in Ştefăneşti wine growing region in the last 5 years.

MATERIALS AND METHODS

The study of the influence of climatic factors on the yield and quality atthree cultivars of grapes of wine 'Feteasca Albă', 'Feteasca Neagră' and 'Feteasca Regală' was conducted in 2016, 2017, 2018, 2019 and 2020, respectively. The study was performed at the experimental field of the National Research and Development Institute for Biotechnology in Horticulture, Ştefăneşti. Experimental vineyard was planted in 2004 with the planting distance of 2.50 m between rows and 1 m within rows. Growing form of vine is double horizontal cordon with trunk height of about 80 cm. Mixed pruning was applied. The set of quantitative and qualitative characteristics was basic in due following indicators: relative coefficient of fertility, absolute coefficient of fertility, relative productivity indices, absolute productivity indices and production, total tritrable acidity (TTA), total sugar content (TSC). Fertility and productivity are biological characteristics, describing agrobiological and technological value of grape cultivars. To calculate the fertility, it was necessary to register buds number on block, total number of shoots, total fertile shoots and inflorescences number. Fertility coefficient was calculated as absolute (Afc) and relative (Rfc). Therefore, Afc= Number of inflorescences/Number of fertil shoots, and Rfc = Number of inflorescences/ Number of total shoots, respectively. The productivity was calculated through absolut productivity indices (Ipa) and relative productivity indices (Ipr),using the following formulas: Ipa = Afc x Gm, Ipr = Rcf x Gm, with Afc – absolute fertility coefficient; Rfc – relative fertility coefficient and Gm – average grape weight. The total acidity was determined by the titrimetric method. The total sugars contentwere evaluated by refractometric method, by measuring the percentage of soluble solidsor refractive index, after prior removal ofalcohol and volatile compounds from wine (which changes the refractive index value). For the statistical interpretation of the results, the data were

included in an Excel database and then statistically interpreted with the SPSS 14.0 program, which uses the Duncan test (multiple range test) for a 5% statistical assurance.

RESULTS AND DISCUSSIONS

Yield quantity and quality is heavily influenced by climate and the prevailing meteorological conditions in production regions (Mirošević and Karoglan - Kontić, 2008). Air temperature exerts dominant influence on vines depending on vine phenological dynamics, and in current climate changing condition, heat regime changes are the most pronounced. We had available for this analysis, daily climatological databases collected from the meteorological platform of the National Research and Development Institute for Biotechnologies in Horticulture, Ştefăneşti, for the experimental period (2016-2020), ascompared to multiannual values (1977-2014).

Monthly average temperatures were used to calculate a set of bioclimatic indices commonlyused in viticulture. During the study period (2016-2020), higher temperatures were recorded, as compared to the multiannual average, especially with regard to the maximum annual temperatures (Table 1). At the same time, the absolute minimum winter temperatures have been harmful to the vine in all the three years of experimentation.

Table 1. The climatic indicators during experimentation period (2016-2020) compared to the multianual average (1979-2015), at INCDBH Ştefăneşti

Climatic indicator	Multianual average (1979-2015)	2016	2017	2018	2019	2020
Average annual temperature, ^{0}C	10.68	12.08	11.94	12.09	12.81	12.23
Average temperature in the growing season, ^{0}C (IV-X)	16.66	14.74	15.06	19.21	18.37	19.93
Average temperature in summer, ^{0}C (VI-VIII)	20.77	23.2	23.13	22.27	22.87	19.35
Average annual minimum temperature, ^{0}C	6.13	5.8	5.42	6.09	6.23	7.8
Absolute minimum temperature, ^{0}C	-22.4	-16.6	-9.6	-18.4	-16.6	-10.9
Average January minimum temperature, ^{0}C	-3.88	-6.2	-9.4	-10.7	-12.7	-10.1
Average annual maximum temperature, ^{0}C	21.74	20.81	20.99	21.09	21.82	23.72
Average July maximum temperature , ^{0}C	28.1	34.8	33.1	32.4	32.5	37.7
Annual total precipitation, mm	725.08	858.2	843.4	831.2	657.6	380
Total precipitation in the growing season, mm (IV-X),	494.33	606.4	631.2	480	444.2	337
The total precipitation in summer (VI-IX)	304.7	338.6	330.4	303.8	258.4	225.4

The vegetation periods of 2016 and 2020 were drier and summer rainfall (VI-IX) was lower than usual except for 2016 and 2017, when the precipitation exceeded the normal values by 33.9 mm, 45.7 respectively. These climatic conditions have led to changes in the development of the grapes' yield and its quality. Table1 describes the climatic indicators during the experimentation period (2016-2020) compared to the multianual average (1979-2015), at INCDBH Ştefăneşti. Table 1 clearly shows that air temperature in Ştefăneşti vine growing region is in constant growth, especially in the summer period. With climate anomalies, expressed through the differences in the values of climate parameters between the multi-year average (1979-2015) and the period from 2016 to 2020, highlighted that the climate in this wine-growing region is rapidly changing. Compared to long-term averages, average annual air temperature was higher with 1.4°C in 2016 was higher in 2017 - 1.26°C, with 1.41°C in 2018, in 2019 with 2.13°C, and in 2020 was with higher that 1.55°C at the level of long-term averages.

Applying the strongest statistical test in order to verify the normality of the distribution (Shapiro-Wilk, W), the values W = 0.942 at Afc, W = 0.967Rfc, W = 0.688 Ipc and

W =0.887 Ipr were obtained which determines the acceptance of normality in the case of all quantitative indicators analysed (table 2). Grape yield per area unit, as an absolute indicator of productivity of cultivars, is conditioned by a number of factors. Among the more important are biological characteristic of the cultivar and environmental conditions in the studied years. The results of histograms of quantitative indicators showed that the yield of grapes was in accordance with the weather conditions.

Table 2. Statistical tests from verification the normality of the distribution of biological indicators in Fetească Albă, Fetească Neagră and Fetească Regală cultivars

Indicators	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Absolute coefficient of fertility (a.f.c)	.119	180	.000	.942	180	.000
Relative coefficient of fertility	.144	180	.000	.967	180	.000
Absolute productivity indices (bunches/shoot)	.328	180	.000	.688	180	.000
Relative productivity indices	.187	180	.000	.887	180	.000
Productions (t/ha)	.110	180	.000	.942	180	.000

a Lilliefors Significance Correction

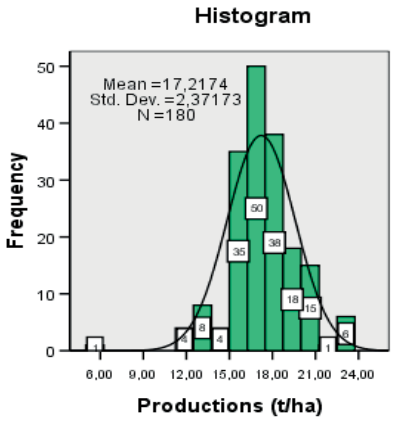

Figure 1. Histogram of class distribution of absolute frequency of the production

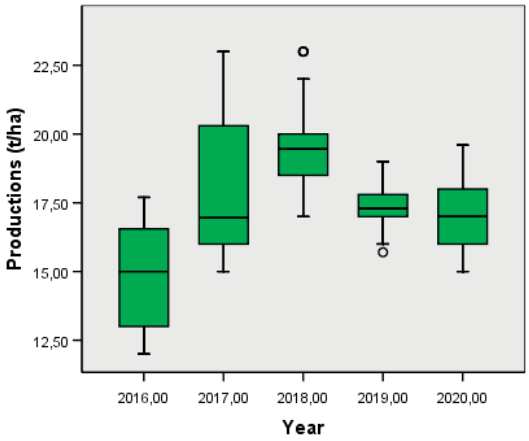

Figure 2. The box plot describes the distribution of yield (tons per hectars) related to the three cultivars

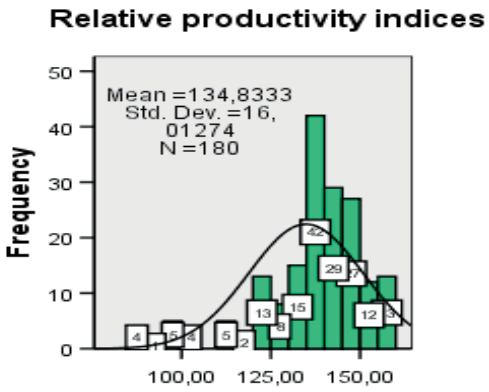

Figure 3. Histogram of class distribution of absolute frequency of the relative productivity index

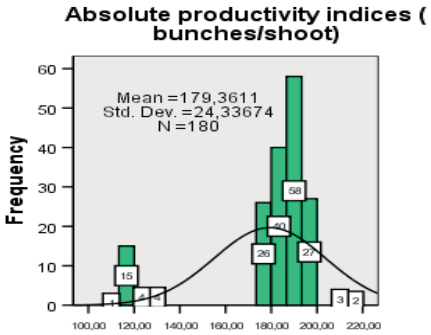

Figure 4. Histogram of class distribution of absolute frequency of the absolute productivity indices

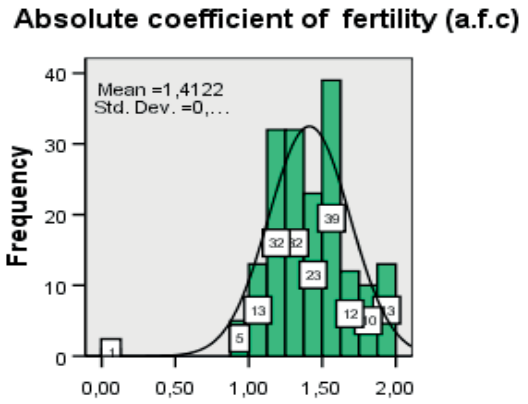

Figure 5. Histogram of class distribution of absolute frequency of the absolute coefficient of fertility

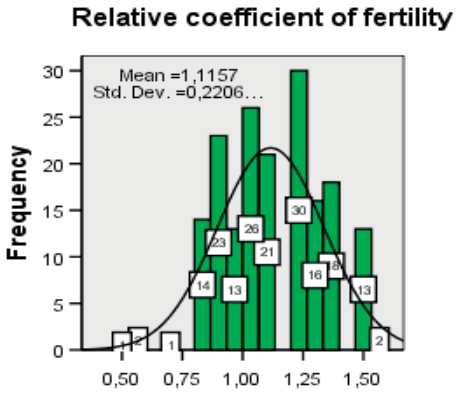

Figure 6. Histogram of class distribution of absolute frequency of the relative coefficienty of fertility

To was determine the grape production the of analysis the between the interaction cultivar andyear of study the Duncan test used (figures 7 and 8). The analysis of the influence of the cultivars on the production ofgrapes, depending on the years analysed, we but into evidence the following results (figure 8):

- Talking into account the average of grape production during the five years of study.
- On the average of the 5 years of study, the largest grape production was recorded in the cultivars 'Fetească Albă' (17.66 t/ha), followed by 'Fetească Neagră', and the lowest production was recorded in the cultivars 'Feteasca Regala' (16.84, t/ha), the differences between them not being statistically ensured (figure 8).
- The highest values of grape production were registered in 2018 (19.59 t/ha), followed by 2017 (17.34t /ha), and the lowest values of this indicator in 2016 (14.71t/ha).

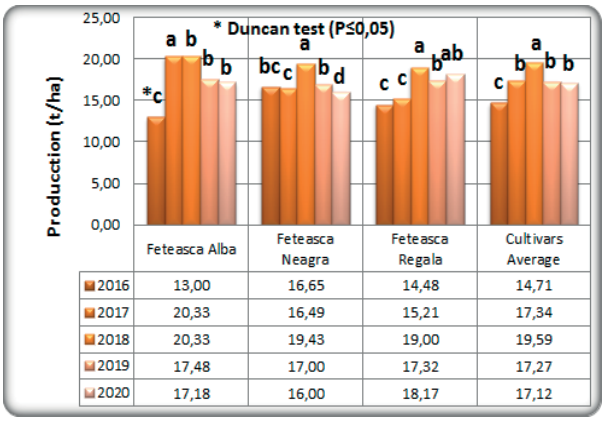

Figure 7. Influence of the production of grapes, depending on the cultivars

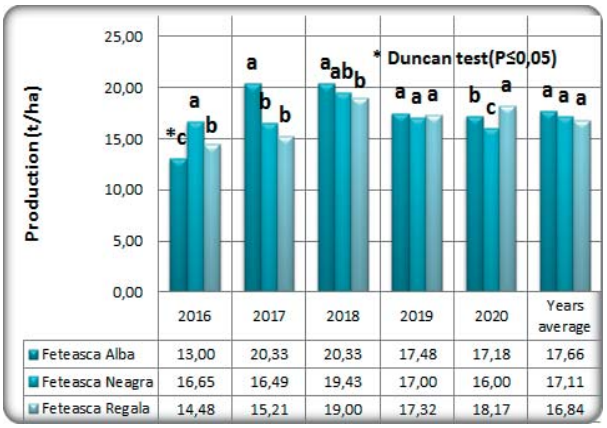

Figure 8. Influence of the cultivar on the production, depending on the years

Wine grape cultivars are usually harvested at a high sugar content, compared to table grapes. According to The International Organisation of Vine and Wine (OIV), resolution VITI 1/2008, grapes with a Brix degree equal to, or above 16, shall be considered ripped (OIV, 2008).Therefore, to determine the qualitative indicators of grape the interaction between of cultivarandyear of study using the Duncan test (figures 9, 10, 11 and 12).

Based on the results shown in figures 9, was performed the highest sugar content of the grapes was measured in 2020 (228,78 g/l) followed by 2019 year, and the lowest in 2017 (196,68 g/l) and 2016, (197,0 g/l), respectively. This is the direct consequence of the variation in weather conditions that prevailed during the experiment period. In 2017 and 2016, with the lowest mean air temperature in vegetation period and the highest amount of precipitation in the vegetation period, the sugar content was lowest. Similar results were reported by Odăgeriu et al. (2012), Antoce (2018) who measured higher sugar content in years with higher mean temperatures in vegetation period. The importance and impact of growing conditions on the yield and quality of grapes indicate Mota et al. (2008).If we analyse the influence of the cultivars on the total sugar content of grapes, depending on the years analysed, we emphasize the following results (figure 10), on the average years, the highest content in total fruit sugar was obtained from the 'Feteasca Regala' (216,25 g/l) cultivar and the lowest values to the 'Feteasca Alba' cultivar (209,64 g/l).

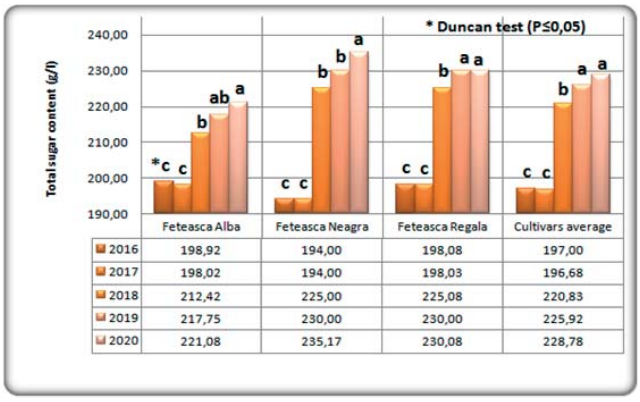

Figure 9. Influence of the total sugar content of grapes, depending on the cultivars

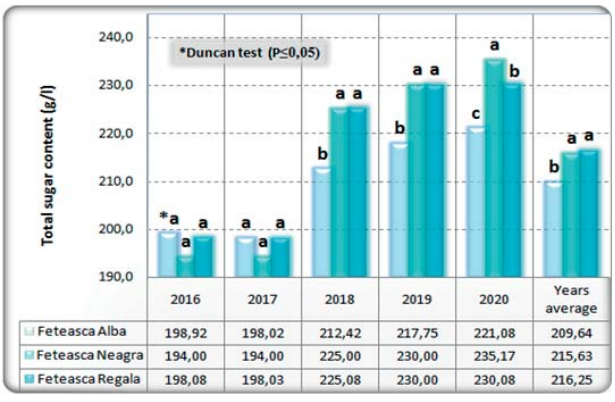

Figure 10. Influence of the cultivar on thetotal sugar content (g/l), depending on the years

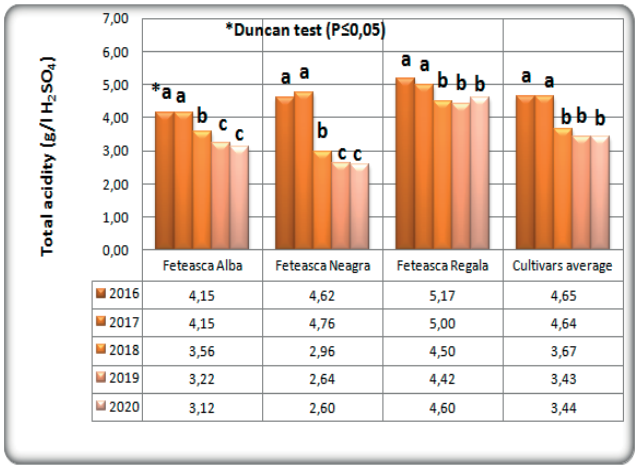

Figure 11. Influence of the total acidity of grapes, depending on the cultivars

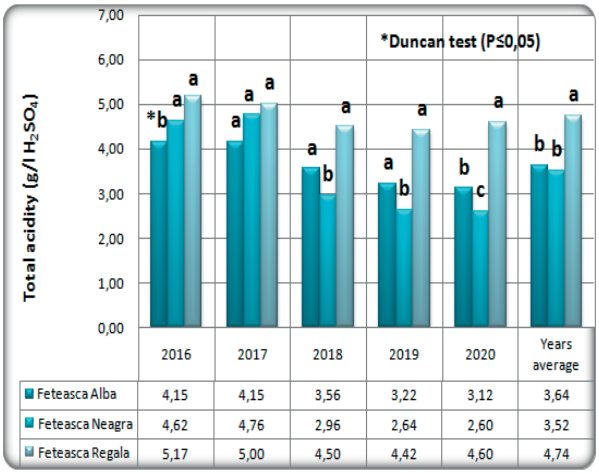

Figure 12. Influence of the cultivar on thetotal acidity (g/l), depending on the years

In these two years total rainfall in the growing season, mm (IV-X), exceeded normal (494.33 mm) by 112.07 mm in 2016 and 136.87 mm in 2017. Average of the cultivars, the highest values of grape acidity were registered in 2016 (4.65 g/l H_2SO_4), followed by 2017 (4.64 g /l H_2SO_4), and the lowest values of this indicator in 2019 and 2020 (3.43 g/l H_2SO_4, 3.45 g/l H_2SO_4, respectively), the differences between them being statistically ensured (figure 11). The low content of grapes in acidity in 2019 and 2020 is due to their higher accumulations in total sugar. The development of grapes maturation in warmer times more than usual, has led to a forced and increased accumulation of sugars, an excessive reduction in acidity, which affects the balance between sugar and acids and requires a careful choice of the harvesting time. The best ratio between the total sugar content of grapes and acidity was registered in 'Feteasca Regala' cultivar. Similar results were reported by Antoce (2018) in five clones of 'Feteasca Neagra' cultivated in regions with higher mean temperatures during vegetation periodwho registered higher sugar content.

CONCLUSIONS

- In terms of climatic conditions, during the entire vegetation period, the year 2018 was most favorable for the culture ofvines in the Ştefăneşti area.
- Significant impact of climatic factors on the amount and quality of yields of this cultivars was put into evidence. However, despite the increasing influence of climate change, there are still very favorable agroecological conditions for growing 'Fetească Albă', 'Fetească Neagră' and 'Fetească Regală' cultivars in Ştefăneşti wine growing region.
- The highest sugar content of the grapes was recorded in 2020 (228,78 g/l) followed by 2019 year, and the lowest in 2017 and 2016, respectively, consequence of the variation in weather conditions in 2017 and 2016, with the lowest mean air temperature and the highest amount of precipitation during the vegetation period, the sugar content was lowest.
- The low content of grapes in acidity in 2019 and 2020 years is due to their higher accumulations in total sugar.
- Increasing accumulation of sugars and an excessive reduction of acidity, which affects the balance between sugar and acidity are the consequence of higher temperature during the period ripening period of the grapes.

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STUDIES REGARDING A VARIETAL ASSORTMENT OF POTTED CHRYSANTHEMUM

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Abstract

***Chrysanthemum* is one of the major crops in the word and it is grown for two basic ways for cut flowers and potted plants, depending of market demand. In the last years the cultivated areas with *Chrysanthemum* have gained a significant growth. Also, due to the progress made in the selection of chrysanthemum, in order to obtain valuable varieties grown in pots, knowledge of characteristics morphology has of major importance, and the enrichment of the existing assortment in our country is the main objective of the growers. The flowers occur in various forms, and can be daisy-like, decorative, pompon or buttons. In addition, many colors are available, such as white, yellow, red, purple and green. The present paper aimed to study the main characteristics of *Chrysanthemum* grown in pots of the following cultivars: `Vanilla`, `Eté Indien`, `Camina Red`, `Pamplona Jogger White`, `Amelia Jogger`, `Avalon Salmon`. The measurements of the experiment were focused on the vegetative growth of the cultivars listed above (plant diameter, circumferences of the plant, height of plant, number of stems/plant, number of branches/stem, number of flowers/plant and the diameter of the flowers) in order to diversify the current *Chrysanthemum* assortment and to promote the cultivation of the most valuable cultivars. The obtained results shown that `Pamplona Jogger White` cultivar registered increases in the case of the diameter, circumference of the plant, and diameter of the flowers. On the opposite side the 'Camina Red' cultivar recorded the largest number of branches/stem and number of flowers/plant and the 'Avalon Salmon' cultivar has the highest values in terms of plant height and number of stems/plant.**

Keywords*:* characteristics, mums, potted plants, cultivars, vegetative growth.

INTRODUCTION

Ornamental plants breeding and cultivation in protected areas offers a great diversity of decorative forms and is an important part of the trade in decorative plants through flowers and leaves (Anton et al., 2007). For more than 2000 years humans have cultivated and improved *Chrysanthemum* plants. Those belong to the oldest ornamental plants of mankind and are still bred and cultivated in Europe (Halmagyi et al., 2004; Larson, 2012). According to estimates, an important place in the top of flowers grown in protected areas was occupied by the chrysanthemum, one of the most representative plant of gardens and landscape planting design in the autumn season. This is due both to its exceptional decorative qualities and to the autumn plant sale period and market requirements typical from September to December. Due to the diversity of shapes and sizes of the inflorescence, the multitude of colors, its suitability for modern homes, make this flower species to be highly appreciated

by the general public.
Kitamura (*Compositae*, *Anthemideae*), originates from the Greek *krus anthemon*, meaning gold flower (Fukai, 1995), originally from China and Japan, known since 1500 BC. as a vegetable plant for the consumption of leaves, without a doubt chrysanthemum is considered today `queen of autumn flowers` (Toma, 2009).
In Europe, the chrysanthemum was cultivated only in 1789, brought from China to France in the form of three varieties, of which only one could be cultivated. The climate of France, provided seed production facilities, allowed the production of various varieties, some of them being preserved even to this day (Rayonnante variety). Under these conditions, the cultivated areas with chrysanthemums expanded both in the field and in greenhouses. Today, the achievements of Europeans in obtaining new varieties, as well as those of improving cultivation technologies are superior to those in the Far East (Barbu, 2008). Also, In Western European countries, the potted chrysanthemum is the traditional plant for decorating graves in late October-early November, when the memory of the deceased is honored (Toma, 2009).
In Romania, chrysanthemum is reported in 1750 as the `autumn daisy`. The first imports of chrysanthemums to be cultivated around Bucharest were made before the First World War, then the assortment increased with the appearance of the first flowers in the capital, Bucharest (1925-1928), gradually numerous flower exhibitions with this species were organized. A significant development in this field is noticed with the establishment of the National Institute of Horticultural Research (1957), when valuable varieties of chrysanthemums were brought to be studied and cultivated for production. Nowadays, chrysanthemums can be admired in landscape arhitecture arrangements or private gardens (Vidraşcu and Teodorescu, 1993), but also in flower shops and floral design using the most cultivated species for the production of cut flowers, both in greenhouse and in the field is *Chrysanthemum* x *hortorum* Hort. (Cantor, 2016). Thus, commercial chrysanthemum cultivars are globally important cut flower and pot plant species usually cultivated by vegetative cuttings (da Silva, 2004). As chrysanthemums have a great impact on the flower market, numerous investigations and scientific researches of physiology, biochemistry, genetics, cellular ultrastructure have been undertaken, in order to know their biology and ways to optimize their growth and productivity and control major diseases and pests. An important contribution, in this sense, has been brought by many researchers from abroad (Zhong Yao Cai, 2008). Regarding breeding and cultivation in protected areas, where specific environmental conditions can be created, the chrysanthemum can be grown all year round, enriching the assortment of flowers from all seasons. Flowering through controlled crops at any time of year, ensuring quite a long durability in vases of 10-14 days as a cut flower, the possibility of using it in various bouquets and floral arrangements or as a potted plant recommends chrysanthemum as the most accepted and valued species (Băla, 2012). As cut flowers, chrysanthemums are useful for decorating interiors in the form of arrangements in large and medium vases, with a note of elegance and sobriety. In general, due to the richness of shapes and colors, chrysanthemums combine with each other, but can also be combined with other flowering species, having special effects. As a cut flower or in pots, chrysanthemums can be used on all occasions, all year round (van Meeteren et al., 2003; Whale, 2011; Hunter, 2012; Vahdati et al., 2012; Buta and Cantor, 2015). All these qualities mentioned before, determine a wide use of chrysanthemums: field culture for obtaining cut flowers or for decorating green spaces (parks and gardens), potted plant, culture in protected areas (greenhouses and solariums) for cut flowers, especially due to the fact that this species is present throughout the year, especially in summer until autumn when frosts appear (Vidraşcu and Teodorescu, 1993).
The areas cultivated with this flower being significant in the Netherlands, Belgium, France,

Colombia, Germany, Italy and Israel (Buta et al., 2013), and based on this fact, chrysanthemums occupying 35% of the total cutflower production in Japan alone, in accordance that cut flowers are extensively used in Japan: 40% as gifts, 25% for commercial facilities, 25% in domestic use, including religious decorations for Buddhist practices, and 10% for educational such as flower arrangement or ikebana (Boase et al., 1997; Kagami and Okamura, 1997; da Silva, 2004). Commercial hybridization to improve cultivars continues today in Europe, Asia and America. Selection is based not only on flower shape and color but also suitability of seedlings for year-round flowering programs and for postharvest qualities (Lim and Shin, 2007). In our country, in the recent years can be observed an increasingly significant development of chrysanthemum culture as potted plants for the decoration of green spaces, gardens, courtyards, balconies and terraces, but especially for the fact that in Romania the flower that ensures the decoration of graves on November is chrysanthemum. Today, more and more private producers are focusing on growing chrysanthemums in pots to be used in the fall. In this regard, the preferance of varieties will be provided according to the period in which we want to have pots with well-developed and flowering plants in a proportion of at least 90% to have a commercial appearance. The high interest of *Chrysanthemum* cultivars used as cut flowers and pot plants in our country, determined to investigate the new assortiment of *Chrysanthemum* grow in pot (Băla and Berecici, 2011).
The aim of the present research paper was to observation of the morphological and biological traits of chrysanthemums cultivars as `Vanilla`, `Eté Indien`, `Camina Red`, `Pamplona Jogger White`, `Amelia Jogger`, `Avalon Salmon` in accordance with the dimensions of the vegetative part of chrysanthemums.

MATERIALS AND METHODS

The study was perfomed for a period of two years (2018-2019) at the didactic greenhouse of the Floriculture belonging to UASVM Cluj-Napoca (Figure 1). Six cultivars of potted *Chrysanthemum* plants were used in the present work, arranged in three randomized repetitions. Each repetition consisted in five plants, a total of 15 plants/cultivar: `Vanilla` – vanilla, `Eté Indien` - orange-yellow, `Camina Red` – red, `Pamplona Jogger White` – white, `Amelia Jogger` – pink, `Avalon Salmon` – coral (Figure 2), and the average of the experiment was used as control.

Figure 1. Location of the experiment (Didactical greenhouse at UASVM Cluj-Napoca)

The potted *Chrysanthemum* plants subjected in our experiment were numbered from 1 to 90. Regarding the study of plant biology, the following observations and measurements were established: diameter, circumference of the plant, height of plant, number of stems/plant, number of branches/stem, number of flowers/plant and the diameter of the flowers. Periodically chemical treatments were applied against *Puccinia chrysanthemi*, *Oidium chrysanthemi* and aphids.

Figure 2. *Chrysanthemum* cultivars

MATERIALS AND METHODS

The *Chrysanthemum* cultivars were planted in three repetitions, according to the randomized block method. The data were interpreted statistically by calculating the average of experience, and the differences between the variants were established by analyzing the variance and using the Duncan test (Ardelean et al., 2005).
In our experiment, observations were made in order to establish the dimensions of the vegetative characteristics of chrysanthemums . The main observations were: diameter of the plant, circumference of the plant, plant height, number of stems/plant. The observations regarding the floral elements were made on number of stems/plant, number of branches/stem, number of flowers/plant and the diameter of the flowers. These observations were made at full maturity of the flowers, not taking into account those previously destroyed.

RESULTS AND DISCUSSIONS

To identify the possible differences, measurements were made on the vegetative plant structure and on the vigor of the cultivars, comparing with an average of the character studied on the whole group.

Under our experimental conditions, the highest increase at the diameter of the plants were measured at the `Pamplona Jogger White` cultivar which was 51.00 cm and the second was the `Camina Red` with 43.33 cm, which were statistically supported (Table 1). While the `Ete indiene` have registered the smallest increases in the diameter of the plant, which was just 31.00 cm.

Table 1. Diameter of the *Chrysanthemum* plants

Variants	Plant diameter (cm)	% compared to control	Differences compared to control	Significance of differences	Duncan test
Average (Control)	40.50	100.0	0.00	Mt.	A
Vanilla	41.67	102.9	1.17	-	C
Ete indiene	31.00	76.5	-9.50	000	A
Camina Red	43.33	107.0	2.83	***	D
Pamplona Jogger White	51.00	125.9	10.50	***	E
Amelia Jogger	34.33	84.8	-6.17	000	B
Avalon Salmon	41.67	102.9	1.17	-	C
LSD (p 5%) 1.33; LSD (p 1%)1.89; LSD (p 0.1%) 2.74					

The highest increase in the circumference of the shrubs was registered at the `Pamplona Jogger White` cultivar which was 130 cm, and the smallest circumference was 72.33 cm, measured at the `Ete indiene` *Chrysanthemum* cultivar (Table 2). The measurements were statistically demonstrated by the Duncan test.

In the case of experimental conditions the smallest growth of mums was registered at the `Vanilla` cultivar (24.67 cm), followed by the `Pamplona Jogger White` with a 26.33 cm (Table 3). The highest chrysanthemum plant was `Avalon Salmon` cultivar, with a high of 33.67 cm, followed by `Camina Red` cultivar with a difference of just 1 cm.

Table 2. Circumference of the *Chrysanthemum* cultivars

Variants	Circumference of the plant (cm)	% compared to control	Differences compared to control	Significance of differences	Duncan test
Average (Control)	99.06	100.0	0.00	Mt.	A
Vanilla	93.67	94.6	-5.39	000	B
Ete indiene	72.33	73.0	-26.72	000	A
Camina Red	103.67	104.7	4.61	***	D
Pamplona Jogger White	130.00	131.2	30.94	***	E
Amelia Jogger	95.33	96.2	-3.72	000	B
Avalon Salmon	99.33	100.3	0.28	-	C
LSD (p 5%) 1.68; LSD (p 1%) 2.39; LSD (p 0.1%) 3.47					

The `Avalon Salmon` have registered the most number of stems/plant (10 stems/plant), it was followed by the `Camina Red` cultivar with a number of 9 stems/plant (Table 4), the data is statistically supported by the Duncan test.

Table 3. *Chrysanthemum* plant height

Variants	Plant height (cm)	% compared to control	Differences compared to control	Significance of differences	Duncan test
Average (Control)	29.28	100.0	0.00	Mt.	A
Vanilla	24.67	84.3	-4.61	000	A
Ete indiene	30.33	103.6	1.06	-	D
Camina Red	32.67	111.6	3.39	***	E
Pamplona Jogger White	26.33	89.9	-2.94	00	B
Amelia Jogger	28.00	95.6	-1.28	-	C
Avalon Salmon	33.67	115.0	4.39	***	E
LSD (p 5%) 1.55; LSD (p 1%) 2.20; LSD (p 0.1%) 3.18					

Table 4. Number of stems/plant of *Chrysanthemum* cultivars

Variants	Number of stems/plant	% compared to control	Differences compared to control	Significance of differences	Duncan test
Average (Control)	7.67	100.0	0.00	Mt.	A
Vanilla	6.00	78.3	-1.67	000	A
Ete indiene	7.33	95.7	-0.33	-	B
Camina Red	9.00	117.4	1.33	**	C
Pamplona Jogger White	7.67	100.0	0.00	-	B
Amelia Jogger	6.00	78.3	-1.67	000	A
Avalon Salmon	10.00	130.4	2.33	***	D
LSD (p 5%) 0.66; LSD (p 1%) 0.95; LSD (p 0.1%) 1.37					

The number of branches/stem (Table 5) was situated between 5.00 to 9.00 branches per stem. Regarding the number of branches a relative reduction was observed at the the `Vanilla` cultivar (5.00) compared to the control. Although the highest number of branches/stem was determined at the `Camina Red` cultivar (9.00) compared to the control.

Table 5. *Chrysanthemum* number of branches/stem

Variants	Number of branches/stem	% compared to control	Differences compared to control	Significance of differences	Duncan test
Average (Control)	7.00	100.0	0.00	Mt.	A
Vanilla	5.00	71.4	-2.00	000	A
Ete indiene	6.00	85.7	-1.00	00	B
Camina Red	9.00	128.6	2.00	***	F
Pamplona Jogger White	8.00	114.3	1.00	**	E
Amelia Jogger	7.33	104.8	0.33	-	D
Avalon Salmon	6.67	95.2	-0.33	-	C
LSD (p 5%) 0.58; LSD (p 1%) 0.82; LSD (p 0.1%) 1.17					

Considering the number of flowers/plant the best results were obtained at the `Camina Red` cultivar, with an average number of 334.67 flowers/plant. On the opposite side was the `Vanilla` *Chrysanthemum* cultivar, with an average number of 93.33 flowers/plant. The results of this measurements were statistically demonstrated (Table 6.).

Table 6. The number of flowers/plant *of Chrysanthemum* cultivars

Variants	Number of flowers/plant	% compared to control	Differences compared to control	Significance of differences	Duncan test
Average (Control)	201.72	100.0	0.00	Mt.	A
Vanilla	93.33	46.3	-108.39	000	A
Ete indiene	211.67	104.9	9.94	-	D
Camina Red	334.67	165.9	132.94	***	E
Pamplona Jogger White	192.33	95.3	-9.39	-	C
Amelia Jogger	170.67	84.6	-31.06	00	B
Avalon Salmon	207.67	102.9	5.94	-	D
LSD (p 5%) 15.31; LSD (p 1%) 21.77; LSD (p 0.1%) 31.52					

When comparing the mumscultivars flowers diameter, an increase can be observed at the `Pamplona Jogger White`, with an average of 5.57 cm, on the opposite side the `Amelia Jogger` cultivar was placed, with an average diameter of 3.13 cm. In Table 7 it can be observed that between the cultivars there are statistically significant differences reported, however the diameters are almost similar at each cultivar.

Similar results regarding the morphological characteristics were obtained by calvalho *et al*.(2020), at the mini chrysanthemum varieties. According to uddin *et al*., (2015) and ona *et al*., (2015) the *chrysanthemum* cultivars showed a wide range of growth and flowering characteristics, which are preferable traits and objectives for the breeders. There are strong evidence that morphological parameters clearly indicate disticntness among the mums varieties (banerji *et al*., 2012; suvija *et al*., 2016), like shrubs diameter; circumferences of the shrubs; shrubs height; increment in the number of stems/plant, branches/stem, flowers/plant; diameter of the flowers.

Table 7. Flowers diameter of the *Chrysanthemum* cultivars

Variants	Diameter of the flowers (cm)	% compared to control	Differences compared to control	Significance of differences	Duncan test
Average (Control)	4.46	100.0	0.00	Ct.	A
Vanilla	4.13	92.7	-0.33	00	B
Ete indiene	5.27	118.1	0.81	***	D
Camina Red	4.27	95.6	-0.19	0	BC
Pamplona Jogger White	5.57	124.8	1.11	***	E
Amelia Jogger	3.13	70.2	-1.33	000	A
Avalon Salmon	4.40	98.6	-0.06	-	C
LSD (p 5%) 0.17; LSD (p 1%) 0.25; LSD (p 0.1%) 0.36					

CONCLUSIONS

Based on the present study results can conclude that:

The diameter of the plants registered the highest value at the `Pamplona Jogger White` cultivar, and the lowest at `Ete indiene`. In the term of the plant circumferences the highest was determined at the `Camina Red` and `Pamplona Jogger White` cultivars. In the case of the plant height `Avalon Salmon` have registered the biggest growth, followed by `Camina Red`. The results concerning the number of stems/plant `Avalon Salmon` showed higher increases compared to the other cultivars. The cultivar `Camina Red` recorded the most

increses in the number of branches/stem. `Pamplona Jogger White` was the chrysanthemum cultivar with the highest flower diameter observed in the experiment.
The data presented above shown that the `Pamplona Jogger White` and `Camina Red` are the most productive and decorativecultivars of those studied. The potted chrysanthemum cultivars which showed higher morphological characteristics are recommended to be promoted and cultivated in the Romanian area, and due to this the mums breeders have a wide range of choices to make.

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POSTHARVEST

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EFFECT OF DEFICIT IRRIGATION ON QUALITY INDICATORS OF APRICOT FRUITS AFTER HARVESTING AND STORAGE

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ABSTRACT

Trees irrigation is one of the major activities because the fruit production is proportional to water use. The current decrease of water resources leads to the urgent need to adopt a strategy which could be applied to efficiently utilize water without affecting the growth, yield and fruit quality. Therefore, deficit irrigation is an alternative. The crop studied was apricot, 'Orizont' cultivar, 13 years old, grafted on the 'Constanta 14' rootstock. The planting distance was 4 m between the rows and 5 m between trees on the row. The split-plot experiment described here is mono-factorial with irrigation strategy having three graduations. The irrigation regime consists of a fully irrigated treatment (b1, non-stressed) according to the irrigation needs (100% of ETc = ETo x Kc), a deficit irrigation treatment (b2) irrigated with half the amount of water in b1 (50% of ETc), and a control, non-irrigated treatment (b3). These plots comprised three adjacent fruit tree rows, with the central row containing five trees for measurements and observations. This research aims to study the effects of deficit irrigation on some quality indicators of apricot fruits after harvest and storage. Fruits in the b3 treatment were much firmer, followed by the fruits from the b2 treatment. The study suggests that moderate deficit irrigation can be profitable for enhancing key fruit quality characteristics.

Keywords: *Prunus Armeniaca L.*, climate conditions, soil water potential, fruit firmness, ascorbic acid

INTRODUCTION

Drought is one of the main serious problems for fruit production. Therefore, improving water efficiency is a major goal for sustainable fruit growing. Deficit irrigation (DI) is a technique that irrigates the entire root zone with less evapotranspiration and leads to reduce the irrigation water use with maintaining farmers net profits (Hoffman et al., 1990). The effects of water deficit on fruit quality depend on the intensity and duration of the water stress period and on the sequence in which the water deficits occur, as well as on cultivar (Castel and Buj, 1990). In general, the fruit yield reduced in DI system by size and weight reduction of fruit, but quality parameters, such as sugars, ascorbic acid, and anthocyanin contents in fruit increased by water restrictions (Roccuzzo et al., 2014).
Apricot fruits are popular worldwide owing to their high nutritional value and delicious flavor, which not only can be used as fresh fruit but also has a high importance as processed product (Moradinezhad and Jahani, 2016). Fruits are not always consumed immediately

after harvest and are therefore held in cold storage, refrigeration is widely used to delay ripening and control fruit decay (Goto et al, 2011). The ability to maintain fruit quality is defined in terms of appearance (color, absence of degradation signs or physiological diseases) and texture (firmness, juiciness).
This research aims to study the effects of deficit irrigation on some quality indicators of apricot fruits after harvesting and storage.

MATERIALS AND METHODS

Study area. The studied orchard is located in village Agigea (44º05' Northern latitude and 28º37' Eastern longitude), Dobrogea region, Romania. The experiment location has an average altitude of 30 m and is situated about 2 km from the Black Sea.The mean annual temperature in the Agigeavillage was 12.4°Cand total annual rainfall was 489.6 mm (40-year average, i.e. 1975–2015 period).

Experimental design and irrigation application. The split-plot experiment described here is mono-factorial with irrigation strategy having three graduations. The apricot tree (*Prunus armeniaca L.*) was selected for this study because is representative for this region. The biological material is representedby 'Orizont' cultivar omologated in 2004.
Climatic data were recorded by an automatic weather station (WatchDog Weather Station 2000, Spectrum Technologies Inc., Aurora Illinois, USA) by a 1-h step. These data were periodically transferred to a laptop and processed as diurnal means and used in calculations.
The soil is a calcaro-calcic chernozem (*World Reference Base for Soil Resources, 2006) with a loamy texture and alkaline pH, a proper soil structure and high fertility in topsoil (0-60 cm). Land slop is between 1.0 and 3.0% and soil bulk density around 1.20 g cm^{-3}.
The study was carried out during two years (2016÷2017). The fruit trees were planted in spring 2004, in a 4m x 5m layout. The studied plots comprised three adjacent fruit tree rows with the central row containing five trees for measurements and observations. The canopy shape was a classic vase and the soil management systems was clean cultivation both between tree rows and in the row.The irrigation regime consisted of a fully irrigated treatment (b1) according to the irrigation needs (100% of ETc = ETo x Kc, Penman-Monteith method, Allen et al., 1998) as previously described for the region by Paltineanu et al. (2007), a deficit irrigation treatment (b2) irrigated with half the amount of water in b1 (50% of ETc), and a control, non-irrigated treatment (b3). Irrigation application was usually carried out in b1 when soil water content (SWC) was about to reach the mid-interval between field capacity (FC) and wilting point (WP). The watering method used was drip irrigation. The dripper spacing was 0.6 m and the dripper discharge about 2.0 Lh^{-1}. The irrigation period lasted from July to August in 2016 and June to September in 2017.In 2016, we applied just four watering, with 20 mm in b1 and 10 mm in b2, totaling 80 mm in b1 and 40 mm in b2, respectively. However, there were nine irrigation applications during the dryer period in 2017 each of 20 mm in b1 and 10 mm in b2, totaling 180 mm and 90 mm, respectively. No water was applied in b3.

Soil water content measurements. Soil water matric potential (SWP) was measured with Watermark resistance blocks (6450 Watermark Soil Moisture Sensor) installed for each fruit tree at four depths: 20, 40, 60 and 80 cm at a 150 cm distance from the tree trunk. The sensors were placed on the same vertical line at 45° angles below horizontal according to the method described by Paltineanu and Howse (1999). These data were recorded by WatchDog dataloggers (WatchDog Model 1650 Data Logger, Spectrum Technologies) and

downloaded periodically by a laptop. The relationships between SWP measured with the Watermark sensors and SWC measured gravimetrically were previously determined from field data (Paltineanu et al., 2011b); these relationships were then used to transform soil water matric potential readings into SWC values during the experiment.

Fruits and determining fruit quality. Apricot fruits were obtained at the Research Station for fruit Growing (RSFG) Constanţa, located in Agigea, Romania, in a conventional orchard. Apricots were harvested in the first 10-day periodof July. After harvesting and storage (warm storage: 7 days at 18-20°C, 70% relative humidity; cold storage: 14 days at 10-12°C and 4-6°C, 90% relative humidity), firmness measurements, dry soluble substance and ascorbic acid (vitamin C) were made at fruits in the treatments studied (Figure 1). Measurements were carried out ina number of five fruit/treatment, each fruit being penetrated in three points in the equatorial zone. Firmness determination was carried out with OPD table penetrometer, measuring the penetration depth of the conical needle into the fruit pulp expressed in penetrometer units (1UP = 0.1mm). The soluble solids concentration has been determined by refractometry. The refractive index of the product is influenced by the presence of other solutes, for example, organic acids, minerals and amino acids. Determination of ascorbic acid (vitamin C) is based on the extraction of the sample with oxalic acid and titrating with 2.6 dichlorophenol indophenol in excess.

a) b) c) d)

Figure 1. Fruits after harvesting and storage: a) After harvesting, b) Warm storage at 18-20°C, c) Cold storage at 10-12°C and d) Cold storage at 4-6°C

Data analyses. SPSS 14.0 software and Microsoft Office Excelwereused for the analysis of variance and various calculations for fruit quality properties. Different letters in the graphs indicate significant differences for the probability (P) ≤ 0.05 according to Duncan`s multiple range test.

RESULTS AND DISCUSSIONS

Climate conditions. For experimental period (2016÷2018), the monthly average air temperatures ranged from 13.7 to 25.1°C from April to September, with mean air temperature value of 20.5°C. No relevant temperature variations were recorded compared with mean multiannual of the area, 19.6°C, respectively (Table 1). Climatic data were recorded by an own automatic weather station (WatchDog Weather Station 2000, Spectrum Technologies Inc., Aurora Illinois, USA).

In the study period, the mean annual rainfall amount was 191.2 mm, versus 272.9 mm for long-term (Table 2). The period of experiment (2016÷2018) was considered as a relatively normal period, showing however atendency of soil moisture depletion.

Table 1. Mean, maximum and minimumair temperature (°C) from April to September over a 3-years period versus the long-term, 1975 ÷ 2015

Climatic element	Air temperature values from 3 years period (2016-2017-2018), °C						
	April	May	June	July	August	September	Mean for April-September period
Mean air temperature, T_{med} (°C)	13,7	18,4	21,8	23,9	25,1	20	20,5
Mean maximum air temperature, T_{max} (°C)	21,3	25,3	28,7	29,8	32,9	27	27,5
Mean minimum air temperature, T_{min} (°C	6,1	11,5	14,8	18	17,3	13	13,5
Mean multiannual temperatures (°C), (40-year average, i.e. 1975–2015 period)	10,9	16,5	20,8	25,1	24,5	19,9	19,6

Table 2. Cumulative rainfall (mm) from April to September over a 3-years period versus the long-term, 1975 ÷ 2015

Climatic element	Rainfall values from 3 years period (2016-2017-2018), mm						
	April	May	June	July	August	September	Mean for April-September period
Rainfall, R (mm)	16,4	44,4	56,9	63,5	9,2	0,8	191,2
Mean multiannual rainfall (mm), (40-year average, i.e. 1975–2015 period)	33	46	43,3	65,5	42,6	42,5	272,9

Soil water content (SWC) during in irrigation period. In 2016, following the application of four watering, the dynamics of soil water content is illustrated in Figure 2. As in previous years, SWC values in both b1 and b2 varied between FC and management allowed depletion (MAD). After applying the last watering, the SWC values were closer to FC. In b3, SWC values were within thehalf interval between MAD and WP.

In 2017, following the application of nine irrigation applications, the dynamics of soil water content is illustrated in Figure 3. In the irrigated treatments, the SWC values oscillated in the range between FC and MAD, the SWC values of b1 being much closer to FC. In b2, SWC values were to half interval between FC and MAD, with values closer to MAD in the 80 cm depth.In b3, SWC values were within thehalf interval between MAD and WP, with values approaching to WP at the end of growing season.

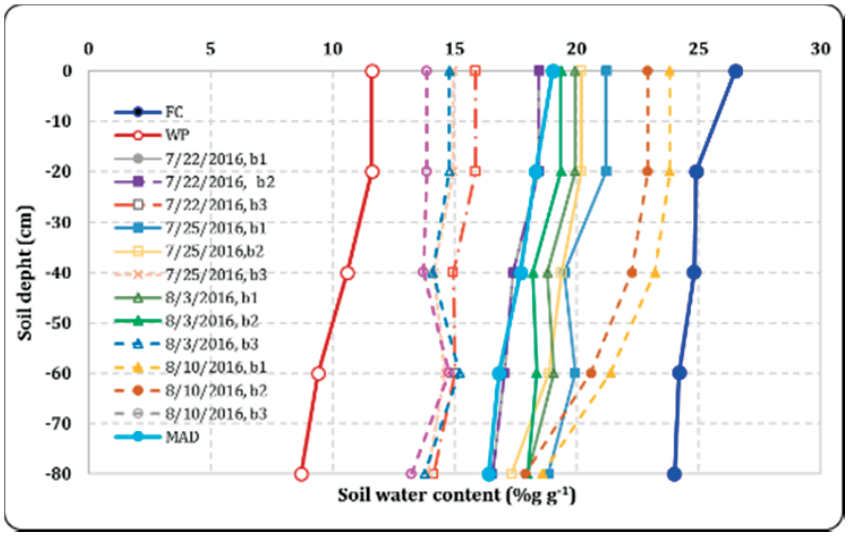

Figure 2. Soil water content (SWC) in experimental plot, Agigea village, Dobrogea- 2016

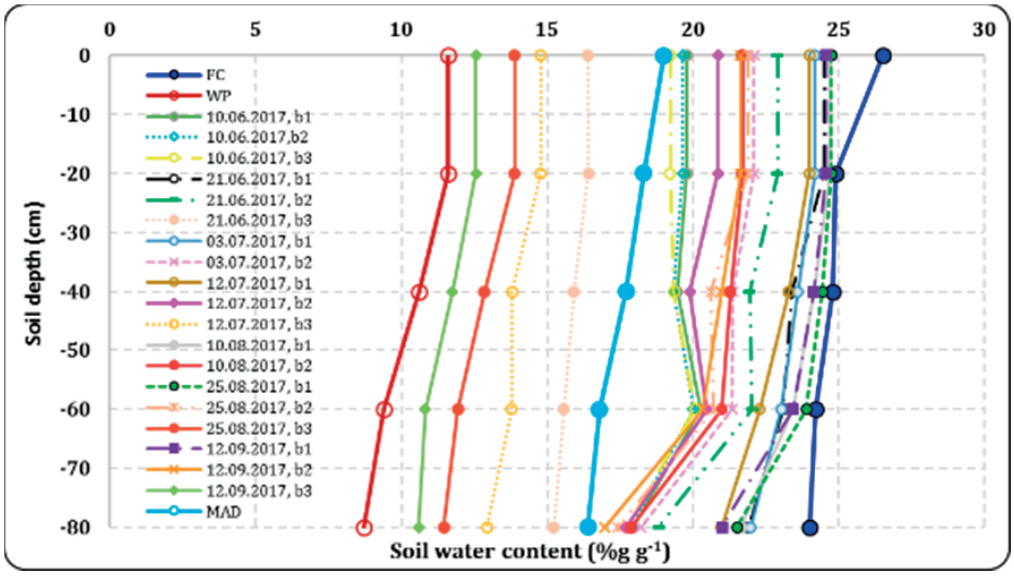

Figure 3. Soil water content (SWC) in experimental plot, Agigea village, Dobrogea-2017

Determining fruit quality after harvesting and storage.

After harvesting and storage, the fruits of experience havebeen subjected to firmness measurements, dry soluble substance and ascorbic acid (vitamin C). After harvesting, apricots had a firmness of 98.89 UP to 109.54 UP (figure 4).After warm storage, apricot fruits had a firmness of 137.80 UP to 154.65 UP, after cold storage, at 10-12°C, apricots had a firmnees of 137.05 UP to 158.70 UP and after cold, at 4-6°C, apricot fruits had a firmness of 123.70 UP to 153.65 UP (figures 5, 6 and 7).

Fruits in the b3 treatment were much firmer, followed by the fruits from the b2 treatment. The smallest firmness was found in b1 treatment.

Figures 4-7 show that there were significant differences between the treatments studied on fruit's fermity, as indicated by different letters accoring to the probability (P) ≤ 0.05 according to Duncan`s multiple range test.

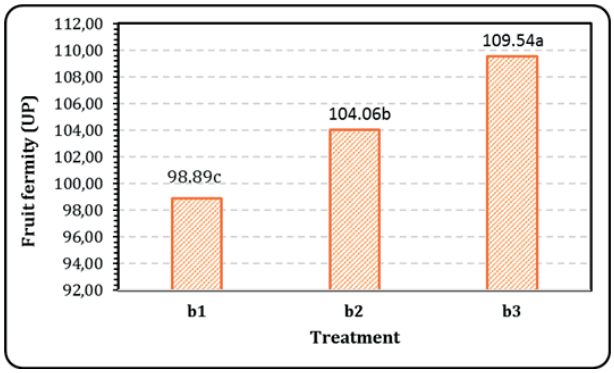

Figure 4. Apricot fruit fermity after harvesting, 2016-2017 period (mean values)

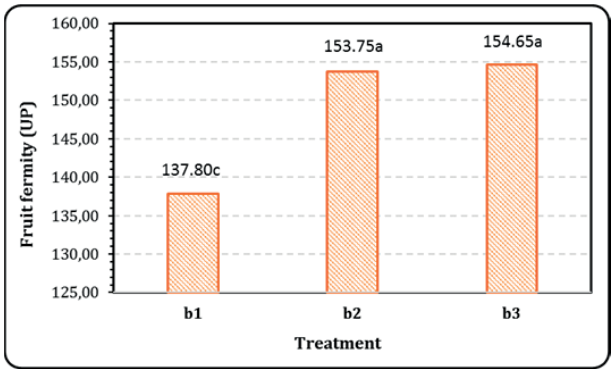

Figure 5. Apricot fruit fermity after warm storage, 2016-2017 period (mean values)

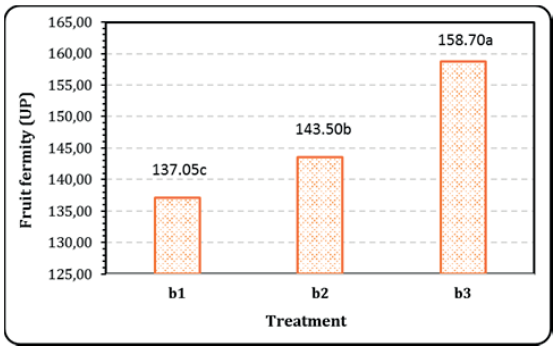

Figure 6. Apricot fruit fermity after cold storage at 10-12°C, 2016-2017 period(mean values)

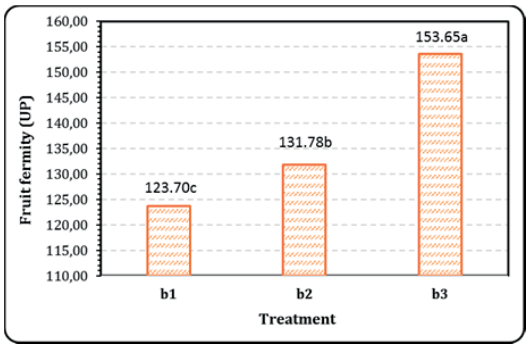

Figure 7. Apricot fruit fermity after cold storage at 4-6°C, 2016-2017 period(mean values)

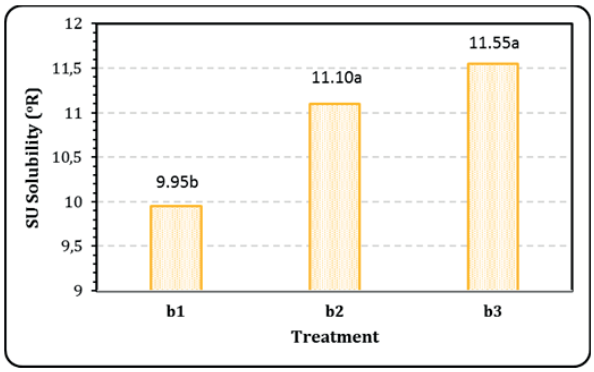

Figure 8. SU Solubility for apricot fruits after harvesting, 2016-2017 period(mean values)

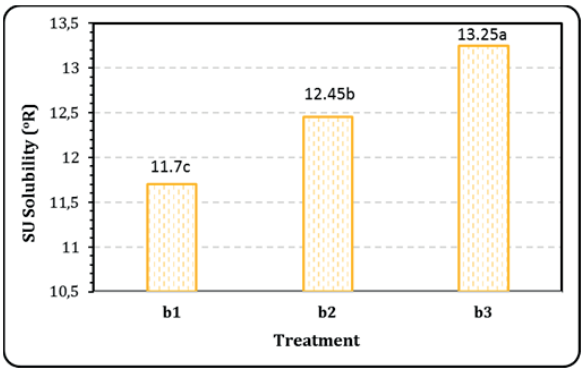

Figure 9. SU Solubility for apricot fruits after warm storage, 2016-2017 period(mean values)

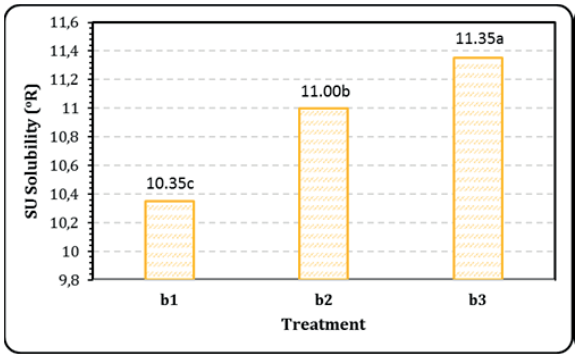

Figure 10. SU Solubility for apricot fruits after cold storage at 10-12°C, 2016-2017 period(mean values)

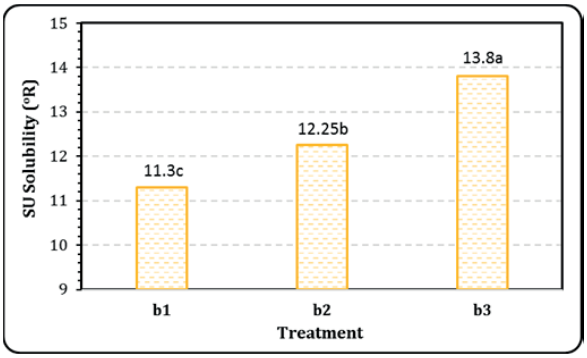

Figure 11. SU Solubility for apricot fruits after cold storage at 4-6°C, 2016-2017 period(mean values)

As with fruit fermity, the dry soluble matter determined on the fruits of the studied treatments had the same trend. After harvesting, the highest value was obtained in b3 treatment, 11.55°R and the lowest value in b1 treatment, 9.95°R, respectively (figure 8). After storage, the highest value was obtained in b3 treatment, 13.8°R -after cold storage at 4-6 °C (figure 11) and the lowest value in b1 treatment, 10.5°R- after cold storage at 10-12 °C (figure 10), respectively.

Figures 8-11 show significant differencesbetween the treatments studied regarding the dry soluble matter of the fruits.

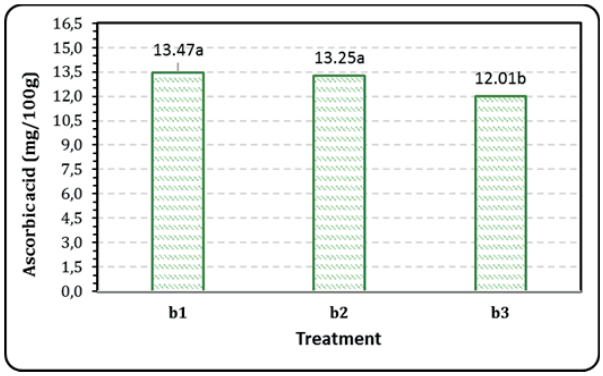

Figure 12. Ascorbic acid for apricot fruit after harvesting, 2016-2017 period(mean values)

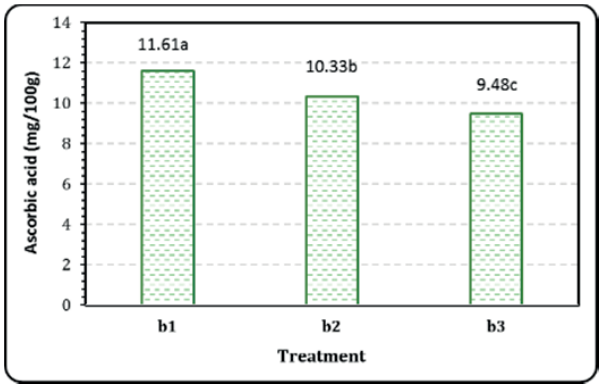

Figure 13. Ascorbic acid for apricot fruit after warm storage, 2016-2017 period(mean values)

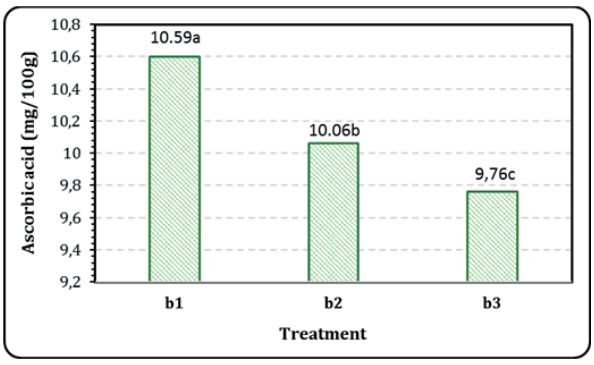

Figure 14. Ascorbic acid for apricot fruit after cold storage at 10-12°C, 2016-2017 period(mean values)

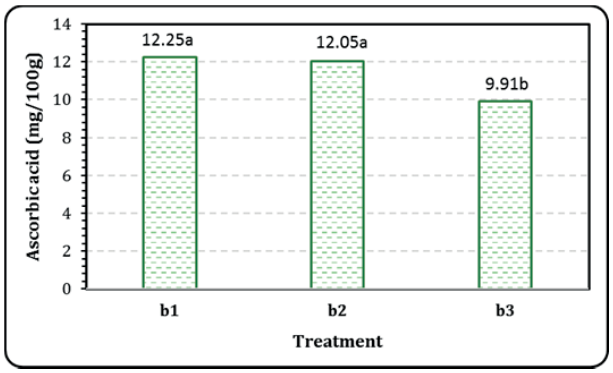

Figure 15. Ascorbic acid for apricot fruit after cold storage at 4-6°C, 2016-2017 period(mean values)

Figures 12-15 show the values of ascorbic acid (vitamin C) of the fruits in the studied treatments. Even if the differences between the treatments were not large, they were signifficantly different; this finding emphasizes again the positive effect of irrigation application in apricot orchards, even if was applied as deficit irrigation.

CONCLUSIONS

Apricots are climateric fruits whose properties declined rapidly after harvest.Therefore, it is recommended to store them in certain environmental conditions in order to maintain fruit quality.

Following the study, the highest values for fruit firmness and dry solubility were obtained in non-irrigated treatment (b3) and in the deficit irrigation treatment (b2), both after harvesting and storage.

In the case of ascorbic acid (vitamin C) of fruits, even if the differences between treatments were not large, they were significantly different, which again emphasizes the positive effect of irrigation in apricot orchards.

Deficit irrigation technique in fruit orchards are indispensable need for preserving tree qualitywater saving and maintaining high the fruit quality.

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POSTHARVEST QUALITY OF STRAWBERRY FRUITS GROWN IN CONVENTIONAL SYSTEM

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ABSTRACT

The objective of the study was to evaluate some postharvest parameters of fruit quality at seven strawberry cvs., ('Alba', 'Clery', 'Coral', 'Magic', 'Premial', 'Queen Elisa', 'Record') grown at RIFG Piteşti, Romania in open field, in the 2016-2018 period. The storage method included three days in normal refrigeration condition (2-4ºC) followed by one day at room temperature (22-24 ºC). The following parameters were determined before and after storage: fruit weight (g), fruit firmness (kgf/cm^2), four color indicators (L*, a*, b*, ΔE) and percent of damaged fruits caused by pathogen. Statistically assured differences were recorded between cultivars. 'Premial' cv. proved the lowest fruit weight loss (0.27 g) and also the lowest fruit firmness loss (0.09 kgf/cm^2) after storage. 'Alba' cv. had the highest percentage of healthy fruits after storage (86.4%). After four days storage both early cvs.were marketable.

Keywords: *Fragaria x ananassa*Dutch.,cultivars, weight,firmness,color,storage.

INTRODUCTION

Strawberries are a highly perishable fruits because of their vulnerability to mechanical damage and sensibility to pathogens, requiring a rapid initial cooling for storage and distribution to the market. Some of fruit qualities traits as general appearance, size, shape, firmness and color can influence the consumer'sdecisionon market (Battino et al., 2019, Temocico et al., 2019, Mazzoni et al, 2020). For example red bright is favorite (Kader 1999; Moshiur Rahman et al., 2014; Moshiur Rahman et al. 2015, Temocico et al., 2017). Because of high metabolic activity, strawberry fruit quality decreases rapidly after harvest (Olias et al., 2000). Fruit storage at ambient temperature makes the management of post-harvest strawberries to be very difficult (Asrey et al., 2004, Ayala-Zavala et al., 2004). Storage of fruits in cold conditions is used on large scale for prolonging the quality. The objective of thisstudy was to evaluate the post harvest quality parameters of seven strawberry cultivars.

MATERIALS AND METHODS

The study was conducted between 2016-2018, on a sample of 500 g of fruit for each studied cultivar 'Alba', 'Clery', 'Queen Elisa', 'Record' (Italian origin) 'Coral', 'Magic', 'Premial' (Romanian origin),at the third harvest of the each year. The field trials were grown in conventional and perennial system, and before planting the following quantities were applied 40kg ha^{-1} N, 40 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O, as basic fertilization.
Straw were used as mulch and the irrigation system used was the sprinkler irrigation type. Soil type prevailing in the field a trial has medium-textured and the heavy-clay soils: clayey-

illuvial luvisols, all showing medium and low humus content. The strawberry fruits were harvested at commercial maturity and the fruits samples were analyzed immediately after picking. The optimum moment for harvesting strawberries was considered when the entire surface was red (Voca et al., 2008).

The fruits were kept for three days in the refrigerator (2-4°C) and one day at room temperature (22-24° C). The following parameters were determined before and after storage: fruit weight (g), fruit firmness (kg/ cm^2), and four color parameters: L*(brightness-darkness), a*(green-red), b*(blue-yellow), ΔE (color evolution during storage) and the percentage of damaged fruit caused by pathogenicmicroorganisms. The fruit weight was determined by measuring the individual fruit with HL-400 digital balance. The fruit firmness was determined on each fruit for each sample with a non-destructive Bareiss HPE II Fffpenetrometerwith a measurement area of 0.50 cm^2.

The color of the fruit was determined by Konica Minolta CR 400 colorimeter, based on Huntel system L*, a*, b* on both sides of the fruit. The color space is organized as a cube. The axis L* represents the brightness, where the maximum value 100 represents white color and the minimum value 0 represents black. a* and b* axes have no specific numerical limits. The positive values for a* indicates red color and the negative values, green color.

The negative values for b* indicates blue color and the positive values, indicates yellow color. The small values of the color indicators L *, a *, b * generally show the darker fruits (Zorrilla-Fontanesi et al. 2011).

The color difference at harvest versus the end of the storage period was determined by the formula: $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ (Faedi et al., 2002). Degradation of the fruits appearance at the attack by the pathogens during storage was visually determined after the storage period. The strawberries where was developed mycelium on the surfaces were considered decayed.

The results were expressed as the percentage decay incidence of the strawberry. Statistical processing of data was conducted using analysis of variance and was used to calculate was Duncan multiple range test, for a probability of error ≤0.05.

RESULTS AND DISCUSSIONS

Weight loss of fruit

The highest values of the fruit weight wereobtainedfor'Record' (17.73 g harvest and 17.24 g after storage, respectively) and the lowest for'Coral'. (10.93 g at harvest and 10.53 g after storage, respectively) Table 1. After the published data by Lovatti and Nuzzi (2009), in Cesena (Italy) 'Alba'showed a fruit weight of 26.0 g at harvest and 24.0 g after storage, consequently a weight loss of 2 g. Even if in our study 'Alba' cv. provedto have a smaller fruit weight because of environment conditions and culture technology, the fruit weight loss of this cv. after storage was 0.48 g (Table 1).

The analysis of fruit weight variation between the two moments of recording data (before and after storage), showed a decrease in the values between cultivars from 0.40% 'Coral' to 1.73% 'Magic' (Table 1).

Firmness loss of fruit

At harvest 'Record'cv.showed the highest value (2.38 kgf/cm^2) of the fruit firmness which is not significantly different versus the others studied cultivars (from 0.12 kgf/cm^2 to 0.62 kgf/cm^2). Table 2 illustrates the value of fruit firmness before and after the storage period and it has found that there were not significant differences between cultivars. The average values recorded for all cultivars decreased with 0.33 kgf/cm^2.

Table 1. Fruit weight of strawberry cultivars

Cultivar	Fruit weight (g) at harvest	Fruit weight (g) after storage	Weight loss (g)
Alba	12.83 c*	12.35 c	0.48
Clery	12.23 c	11.59 c	0.64
Coral	10.93 d	10.53 c	0.40
Magic	16.63 ab	14.90 b	1.73
Premial	16.33 b	16.06 ab	0.27
Queen Elisa	16.13 b	15.33 b	0.80
Record	17.73 a	17.24 a	0.49
Average	14.69	14.00	0.69

*Means within each row followed by different letters are significantly different according to LSD test ($P \leq 0.05$).

Table 2. Fruit firmness of strawberry cultivars

Cultivar	Fruit firmness (kgf/cm^2) at harvest	Fruit firmness (kgf/cm^2) after storage	Firmness loss (kgf/cm^2)
Alba	1.90 ab*	1.72 ab	0.18
Clery	1.90 ab	1.83 ab	0.07
Coral	1.76 b	1.58 ab	0.18
Magic	2.07 ab	1.30 b	0.77
Premial	2.00 ab	1.91 ab	0.09
Queen Elisa	2.26 ab	2.10 a	0.16
Record	2.38 a	1.54 ab	0.84
Average	2.04	1.71	0.33

*Means within each row followed by different letters are significantly different according to LSD test ($P \leq 0.05$).

Surface color stability after storage anddecay incidence of strawberry

The color is an important indicator for determining the quality of fresh products and the changes of the L* a* b* after the storage period are noted in Table 3.

On average for the three years of study and the seven studied cultivars the analysis of the color indicators showed that there were significant differences between the values recorded at harvest and after the storage period.

For L* and a* parameters the differences between the two moments of evaluation (at harvest and after storage) were 0.01 units and 0.05 units, respectively. As average for the three years of study and the two moments of evaluations 'Premial' showed the highest values for three indicators of color, significantly different from all the other cultivars.

Table 3. Fruit colour of strawberry cultivars

Cultivar	Color at harvest			Color after storage			
	L*	a*	b*	L*	a*	b*	ΔE
Alba	31.56 ab*	28.13 b	12.25 b	31.38 ab	28.08 b	12,18 b	2.00 a
Clery	31.60 ab	28.08 b	12.63 b	31.58 ab	28.03 b	12,76 b	0.16 b
Coral	30.19 b	25.12 cd	11.55 b	30.37 b	25.18 bc	11,71 b	0. 87 ab
Magic	31.30 ab	24.01 d	11.96 b	31.38 ab	24.08 c	11,99 b	0.92 ab
Premial	32.42 a	32.73 a	14.54 a	32.33 ab	32.74 a	14.42 a	1.43 ab
Queen Elisa	32.28 a	27.05 bc	12.59 b	32.26 ab	26.90 bc	12.52 b	0.60 ab
Record	32.40 a	25.89 bcd	12.44 b	32.39 a	25.75 bc	12.41 b	0.63 ab
Average	31.68	27.30	12.57	31.67	27.25	12.57	0.94

*Means within each row followed by different letters are significantly different according to LSD test ($P \leq 0.05$).

The lowest values of L* and b* color indicators were registered by 'Coral' for both moments of evaluation (Table 3). 'Coral' were worse noted than 'Premial' according with fruit brightness.

Even if after storage the color evolution (ΔE) showed close values, nevertheless some statistical differences between cultivars occurred, 'Alba' having the highest value (ΔE = 2.00)

Table 4. Color stability and fruit decay after storage

Cultivar	Color stabilityafter storage	% of marketable fruits	Dried calyx %
Alba	medium	86.4	11.4
Clery	medium	75.0	24.1
Coral	medium	56.4	66.3
Magic	medium	50.0	70.2
Premial	hight	54.0	62.4
Queen Elisa	medium	78.4	22.1
Record	medium	70.7	44.2

'Premial' was also noted with higher color stability indicators after storage (Table 4). 'Alba' cultivar was evaluated as having the most marketable fruits (86,4%), taking account also of the lowest percent of dried calyx (11,4%), followed by Queen Elisa with 78,4% and 22,1%, respectively (Table 4).

The nature and intensity of the correlations between the fruit quality indicators

InTable 5 are presented the correlations between studied indicators.

According to the ten indicators studied ΔE was significantly and positively correlated with eight of them, representing color and firmness indicators both at harvest and after storage moments and was negatively correlated with fruit weight (g) for both moments.

The firmness registered at harvest is negatively correlated with three quality indicators studied, and only firmness after storage was negatively correlated with the average fruit weight at harvest.

The fruit weight recorded at harvest was negatively correlated with two fruit quality indicators (ΔE and firmness registered after storage) and was highly significantly positive

with fruit weight measured after storage (r = 0.967). The fruit weight recorded after harvest was negatively correlated only with ΔE (rh = -0.19) and positively correlated with all the other quality indicators.

Table 5. Correlations between fruit quality indicatorsat harvest and after storage (simple Pearson correlation coefficient- r)

The studied character	L* (brightness-darkness) at harvest	a* (green-red) at harvest	b* (blue-yellow) at harvest	L* (brightness-darkness) after storage	a* (green-red) after storage	b* (blue-yellow) after storage	ΔE	Fruit firmness (kgf/cm2) at harvest	Fruit weight (g) at harvest	Fruit firmness (kgf/cm2) after storage	Fruit weight (g) after storage
L* (brightness-darkness) at harvest	1	0.374	0.664 (**)	0.910 (**)	0.339	0.567 (**)	0.063	0.391	0.519 (*)	0.403	0.550 (**)
a*(green-red) at harvest	0.374	1	0.843 (**)	0.350	0.975 (**)	0.836 (**)	0.332	-0.211	0.028	0.300	0.141
b* (blue-yellow) at harvest	0.664 (**)	0.843 (**)	1	0.596 (**)	0.814 (**)	0.931 (**)	0.209	0.026	0.329	0.329	0.409
L* (brightness-darkness) after storage	0.910 (**)	0.350	0.596 (**)	1	0.365	0.623 (**)	0.061	0.335	0.485 (*)	0.350	0.516 (*)
a*(green-red) after storage	0.339	0.975 (**)	0.814 (**)	0.365	1	0.868 (**)	0.365	-0.207	0.029	0.304	0.139
b* (blue-yellow) after storage	0.567 (**)	0.836 (**)	0.931 (**)	0.623 (**)	0.868 (**)	1	0.190	-0.069	0.283	0.267	0.370
ΔE	0.063	0.332	0.209	0.061	0.365	0.190	1	0.015	-0.060	0.127	-0.019
Fruit firmness (kgf/cm2) at harvest	0.391	-0.211	0.026	0.335	-0.207	-0.069	0.015	1	0.592 (**)	0.536 (*)	0.576 (**)
Fruit weight (g) at harvest	0.519 (*)	0.028	0.329	0.485 (*)	0.029	0.283	-0.060	0.592 (**)	1	-0.015	0.967 (**)
Fruit firmness (kgf/cm2) after storage	0.403	0.300	0.329	0.350	0.304	0.267	0.127	0.536 (*)	-0.015	1	0.065
Fruit weight (g) after storage	0.550 (**)	0.141	0.409	0.516 (*)	0.139	0.370	-0.019	0.576 (**)	0.967 (**)	0.065	1

CONCLUSIONS

'Premial' and'Alba' cvs. (early season) were distinguished from all, as follows:'Premial' cv. showed the lowest fruit weight loss (0.27 g) and the lowest fruit firmness loss (0.09 kgf/cm^2) after storage period.
The highest percentage of marketable fruits after storage (86.4%) was recorded by 'Alba'.

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