BEHAVIOR OF VINE VARIETIES WITH BIOLOGICAL TOLERANCE UNDER GIURGIU AREA CONDITIONS

**VIȘAN Valerica Luminița, TAMBA-BEREHOIU Radiana-Maria,
POPA Ciprian Nicolae, DANĂILĂ-GUIDEA Silvana Mihaela**

Abstract. Technological, biochemical and agrobiological studies have been carried out on varieties of vines with biological tolerance in the climatic conditions of Giurgiu area, Romania. The determinations focused on the phenology of varieties, fertility coefficients and productivity indices, the behavior of these varieties on the main diseases of vines, the physical-mechanical characteristics and the technological indices; also, the musts have been analyzed biochemically, including the main flavoring compounds. For the determination of aromatic compounds in grape must, gas chromatography/mass spectrometry methods were used. The studied vine varieties were: *Perla de Zala* and *Moldova* (table vine varieties) and *Radames* (variety of wine). In ecopedoclimatic terms from Giurgiu area, varieties mature in the ages III (*Perla de Zala*) and VI (*Moldova* and *Radames*). The results showed that under the conditions of the analyzed area the varieties entered faster in vegetation and had a slightly higher percentage of fertile shoots than the average recorded in other areas of culture. Fertility coefficients and productivity indices have also been higher in this area. Regarding the tolerance to the main diseases of the vine, the varieties behaved somewhat similarly to other areas of culture, *Perla de Zala* registering a higher degree of attack compared to the other 2 analyzed varieties. Chromatographic gas analysis revealed 28 volatile grape compounds that determine the variety of the analyzed varieties. The results showed a lower number of esters compared to those identified in *vinifera* varieties; in higher concentrations, for all 3 varieties, there were identified: 1-hexanol, caprylic acid, ethyl acetate and 2-pentanones. Among the terpenes, limonene was found in the highest concentration.

Keywords: gas chromatography/mass spectrometry methods, downy mildew, grey mold.

Rezumat. Comportamentul soiurilor de viță-de-vie cu rezistență biologică în condițiile zonei Giurgiu. Au fost realizate studii agrobiologice, tehnologice și biochimice asupra unor soiuri de viță de vie cu toleranţă biologică, în condițiile climatice ale zonei Giurgiu, Romania. Determinările au vizat fenologia soiurilor, coeficienții de fertilitate și indicii de productivitate, comportamentul acestor soiuri la pricipalele boli ale viței de vie, caracteristicile fizico-mecanice și indicii tehnologici; deasemenea, musturile au fost analizate din punct de vedere biochimic, inclusiv privind principalii compuși de aroma varietală. Pentru determinarea compușilor aromatici din mustul de struguri s-a utilizat metoda gaz cromatografică cuplată cu spectrometria de masă. Soiurile de viță de vie studiate au fost: *Perla de Zala* şi *Moldova* (soiuri de masa) și *Radames* (soi de vin). In condițiile ecopedoclimatice ale zonei Giurgiu, soiurile se maturează în epocile III (*Perla de Zala*) și VI (*Moldova* și *Radames*). Rezultatele au arătat ca în condițiile zonei analizate soiurile au intrat mai repede în vegetație și au avut un procent ușor mai ridicat de lăstari fertili decât media inregistrată în alte zone de cultură. Coeficienții de fertilitate și indicii de productivitate au avut, deasemenea valori crescute în această zonă. In ceea ce privește toleranța la principalele boli ale viței de vie soiurile s-au comportat oarecum asemănător ca în alte zone de cultură, Perla de Zala înregistrând un grad de atac mai mare în comparație cu celelalte 2 soiuri analizate. Analiza gaz cromatografică a pus în evidență 28 de compuși volatili din struguri care determină aroma varietală a soiurilor analizate. Rezultatele au arătat un număr mai mic de esteri în comparație cu cei identificați în soiurile *vinifera*; în concentrații mai ridicate, în cazul tuturor celor 3 soiuri au fost identificați: 1-hexanolul, acidul caprilic, ethyl acetatul și 2-pentanone. Dintre terpene, limonenul s-a regăsit în concentrația cea mai ridicată.

Cuvinte cheie: metoda cromatografie gazoasă/spectrometrie de masă, mana viței de vie, putregaiul cenușiu.

INTRODUCTION

Vine varieties with biological tolerance are interspecific hybrids, resulted by hybridization between the European varieties of vine *Vitis vinifera* and hybrids directly producers, coming from the American species (ROBINSON, 1986). The interspecific hybrids divided by generation, in the American old hybrids (first generation), were represented by varieties brought from the American continent, created before the invasion of phylloxera in Europe: *Noah*, *Isabelle*, *Lidia*, *Delaware*, *Othello*, etc.; Euro x American hybrids (second generation, result of crossing between hybrids from the first generation with noble vines): *Seibel 1, Seibel 1000, Terras 20, Rayon d'or*, etc. and the varieties with biological resistance, resulted from multiple crossings between *vinifera* vines with American hybrids (GALET, 1979).

The vine varieties with biological resistance, named by some authors, varieties with biological tolerance, have a lower resistance to the main diseases and pests of vines compared with the old hybrids, but much higher than the *vinifera* noble varieties, thus requiring a much smaller number of phytosanitary treatments (CATTELL, 1979; VIȘAN et al., 2014).

Although their quality does not equal the noble varieties, these varieties have their importance in winemaking, both in improving vine and obtaining green products (juice, alcoholic and non-alcoholic drinks, in different types of food industry).

In many countries in Europe, but especially in the US, they get natural juices of grapes, various foods, as well as nutritional supplements from hybrid grapes (ELFVING, 1992; VIȘAN et al., 2015).

MATERIALS AND METHODS

Vine varieties with biological tolerance *Perla de Zala*, *Moldova* and *Radames* were studied under the ecopedoclimatic conditions of Giurgiu area. The study, conducted over a period of three years, referred to determining phenology variety, fertility, the agrobiological characterization of varieties (percentage of fertile shoots, the fertility coefficients, productivity indices, reaction to major diseases and pests of the vine) and technological characterization of variety (total production grapes/ha, physical and mechanical characteristics of the grapes, technological indices).

The fertility of the shoots was determined by counting the inflorescences, total shoots and fertile shoots. Absolute and relative fertility coefficients were calculated using computation formulas. The productivity of varieties was determined at full grape maturation, using absolute and relative productivity indices (TARDEA *&* DEJEU, 1995).

The average grape weight at full maturity was calculated by weighing 50 grapes and calculating the average. The production of grapes per hectare was calculated according to the production of grapes on the hub and the number of hubs per hectare (OŞLOBEANU, 1980).

In order to assess the behavior at the main diseases of the vine, the degree of attack was determined (DA), calculated according to the relation: (frequency of attack x intensity) / 100 (TOMOIAGĂ, 2006).

Grape musts were analyzed under sugar and total acidity terms (after the standardized methods in effect) and characterized in terms of concentration in volatile compound by gas chromatography method coupled with mass spectrometry.

Gas Chromatography/Mass Spectrometry (GC/MS):

Juice Preparation.

The grapes from the three studied vine varieties were harvested at full maturity. Maturity was estimated by content in sugars (g/L), titrable acidity (g/L sulfuric acid) and berry size. After harvesting, grapes were crushed and pressed with a laboratory winepress. The must was homogenized, filtered and stored at -18°C prior to extraction of volatile compounds.

Extraction of Volatile Compounds.

At the extraction of the volatile compounds, it was used a continuous extractor liquid-liquid. Two hundred milliliters of juice (containing internal standard IS: 1-heptanol) placed in a conical flask, were extracted with 5 mL of distilled dichloromethane (Merck, Darmstadt, Germany) by stirring for 30 min at 0°C and then centrifuged for 15 min at 10000 g. The extract was dried with 4 g sodium sulfate and stored at -18°C until analysis (SEROT et al., 2001; BAEK et al., 1997; VIȘAN et al., 2007).

Gas Chromatography/Mass Spectrometry (GC/MS)

The GC/MS system includes a Hewlett Packard 5890 Series II gas chromatograph and a Hewlett Packard 5971 mass spectrometer. Each extract was injected 1 µl in the spitless mode (200°C injector temperature, 60 sec valve delay) into a capillary column (DB-Wax, 30 m length x 0.32 mm internal diameter x 0.5 µm film thickness). The flow rate of helium (carrier gas) was 1 mL/min. Oven temperature was programmed from 50 to 200°C at a rate of 3°C/min with initial and final hold times of 5 and 50 min (GUTH et al., 1997).

In case of mass spectrometer, the conditions were: ion source temperature: 280°C; ionization energy: 70 eV; mass range: 30-350 a.m.u.; electron multiplier voltage: 2100 V; scan rate: 2.2 sec^{-1}.

The identification of volatile compounds was based on comparison of GC retention indices (RI), mass spectra (comparison with MS spectra database and internal library of the laboratory) and odor properties (TRANCHANT, 1995; LE GUEN et al., 2000).

RESULTS AND DISCUSSIONS

Perla de Zala table variety (synonyms: *Zala Göngye, Egri Csillagok 24, Perle von Zala, Zala Dende)*, genitors (Villard blanc x SV 12 375) x Perla de Csaba (*Vitis vinifera* variety), breaking leaf buds in Giurgiu area around April 20 (Table 1) and has the shortest vegetation of 172 days (Fig. 1) fits in III maturation era.

Regarding the phenology of *Perla de Zala* variety, under the conditions of Giurgiu area, the phenophases begin 5-7 days earlier than in Dealu Mare area, for example, and the vegetation period is reduced by 4-5 days (GRECU, 2010).

Moldova table variety (hybrid obtained in the Republic of Moldova in 1974), genitors *Guzali kara* x *Villard blanc,* breaking leaf buds in Giurgiu area around May 1 (Table 1) has a vegetation period of 182 days and fits in the VI maturation era.

In the studied area, *Moldova* variety behaves similarly to *Perla de Zala*, in the sense that the variety enters the vegetation faster than in case of Dealu Mare vineyard, and the vegetation is shorter by 5 days (182 days compared to 187 in Dealu Mare (GRECU, 2010).

Radames variety, genitors *Traminer Pink* x descendant *Villard blanc* x *Queen vineyards* (obtained by Moldovan St. in 1993) breaking leaf buds in Giurgiu area on April 6 and but has the longest vegetation period (200 days) thus sweeping later, in the VI maturation era.

In the case of *Radames* variety, it is noticeable that in Giurgiu area, the entrance to vegetation is much faster compared to other vineyards, and the vegetation period is shorter by about 9 days compared to Dealu Mare area (MOLDOVAN et al., 1994; GRECU, 2010).

Fertility and productivity of varieties with biological tolerance in the conditions of Giurgiu area:

The 3 analyzed varieties under the ecopedoclimatic conditions of Giurgiu area recorded an average percentage of fertile shoots, ranging between 60.2 and 68.2%, higher than in other areas of culture (VIȘAN et al., 2015).

Table 1. Phenology biological resistant varieties.

Variety	**Average data of phenophases**				
	breaking leaf buds	flowering	first fruits	maturation	fall leaves
Perla de Zala	20.04	1.06	3.08	28.08	18.10
Moldova	1.05	3.06	12.08	1.10	5.11
Radames	6.04	30.05	15.08	8.10	5.11

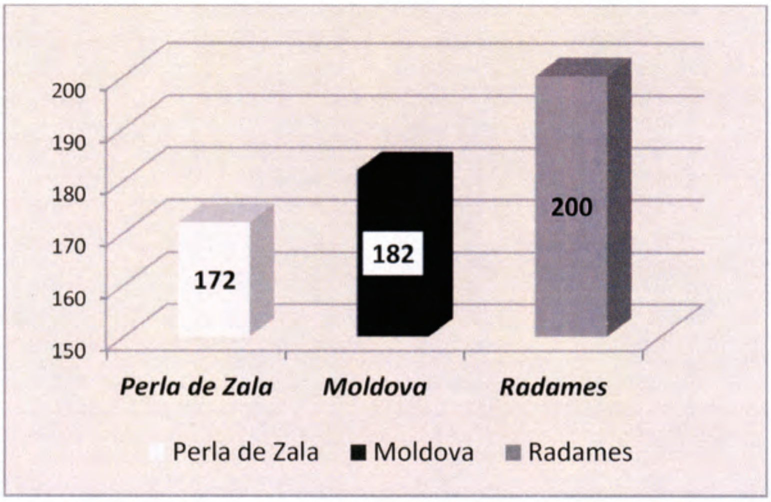

Figure 1. The vegetation period of the vine varieties with biological resistance in the conditions of Giurgiu area (days).

Of the two mass varieties, Moldova has a higher percentage of fertile shoots (68.2%), a higher weight of grape (260 g) and higher productivity indices. Perla de Zala variety has a small grain weight (for a table wine) of 178 g, the lowest percentage of fertile shoots of the analyzed varieties and an average production of 9.6 t / ha (Table 2). Radames variety shows a percentage of 64.5% fertile shoots (Fig. 2) and has an average production of 11 t/ha (Table 2).

Table 2. Fertility and productivity of varieties with biological tolerance.

Variety	**fertility**			**average weight of a grape**	**index of productivity**		**Production of varieties**
	fertile shoots	**fertility coefficients**			**absolute**	**relative**	
	%	**absolute**	**relative**	**g**	**g**	**g**	**t/ha**
Perlă de Zala	60.2	1.7	1.1	178	303	196	9.6
Moldova	68.2	1.9	1.2	260	494	312	13.8
Radames	64.5	1.7	1.0	179	305	179	11

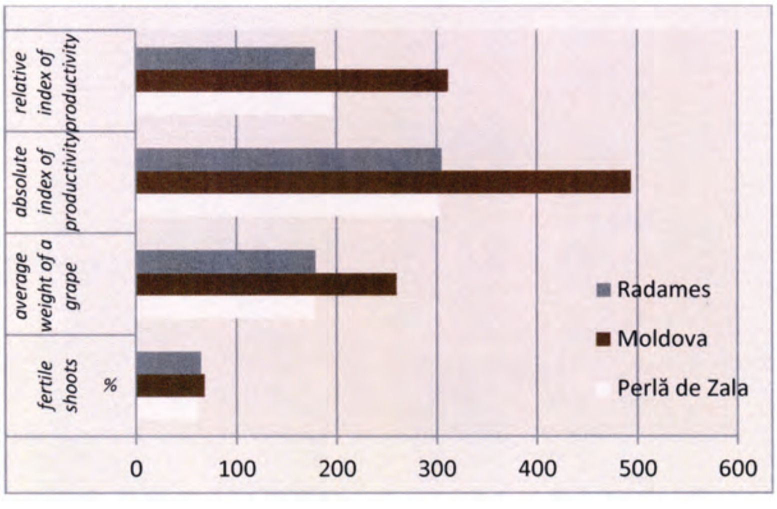

Figure 2. Fertility and productivity of the vine varieties with biological tolerance in the conditions of Giurgiu area.

Regarding the behavior of the varieties with biological tolerance to the main diseases of the vine, it can be noted that is a big difference between the three hybrids, Perla de Zala having a lower resistance to most diseases, the degree of attack ranging between 4% (downy mildew) and 12% (powdery mildew on the grapes). Also Perla de Zala showed the highest degree of mold attack. The hybrids of Moldova and Radames recorded a low degree of attack, especially in downy mildew and grey mold (Fig. 3).

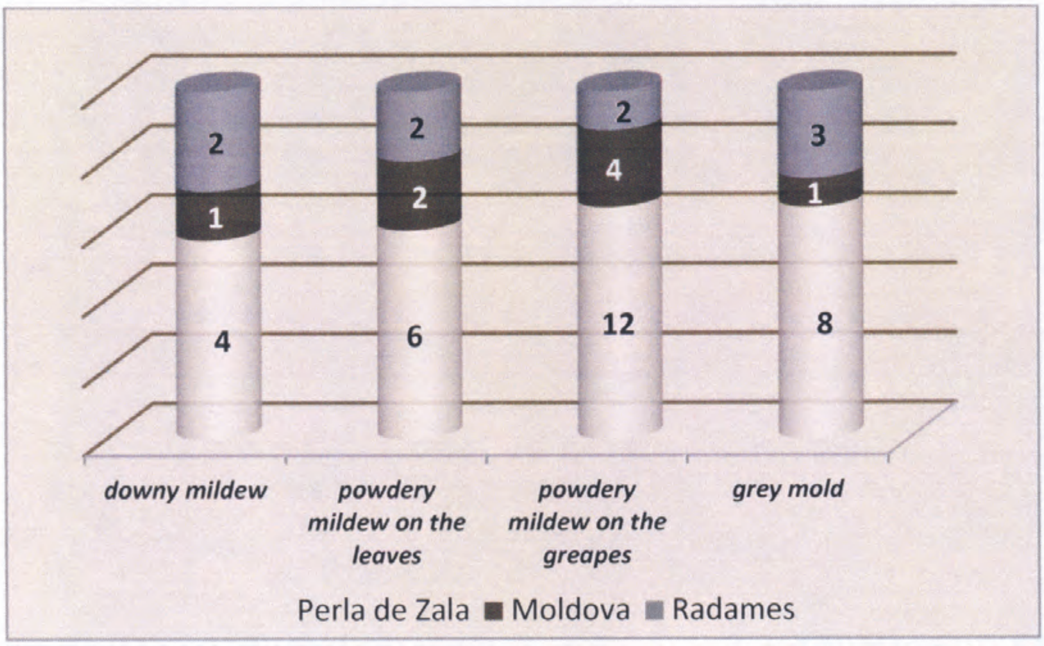

Figure 3. Behavior of vine varieties with biological tolerance to major diseases and pests of the vine (attack degree %).

However, from the point of view of disease tolerance, the hybrids behaved better than in other studied areas, the degree of attack being lower for all diseases (Fig. 4). Physical-mechanical characteristics and technological indices are presented in Table 3.

At the table varieties Moldova shows a grain weigh of 975 g and in terms of yield indices Radames recorded the lowest value of 1.6.

In terms of sugar content, *Perla de Zala* accumulates a high amount of sugars, of 183 g/L, but it has a glucoacidimetric index of 42, higher than the optimal value. *Moldova* variety recorded the lowest sugar content (158 g/L), but a normal glucose index of 32.9. *Radames* variety has moderate sugar content, especially for a variety of wine and a high acidity of 5.8 g/L of sulfuric acid.

As for the sugar content of grapes, under the same climatic conditions all three varieties recorded slightly higher values than Dealu Mare (GRECU, 2010; VIȘAN et al., 2014).

Table 3. The physico-mechanical and technological indices of biological resistant varieties.

Characteristics	**Variety**		
	Perla de Zala	*Moldova*	*Radames*
	Mechanical composition / kg grapes (g)		
Grain weight	972	975	965
Weight of must	759	725	632
Skin and pulp	172	218	288
Rahis	28	25	37
Seeds	41	32	45
Marc	241	275	370
The composition of 100 grains (g)			
grain weight	231	390	160
Skin weight	34	46	36
pulp weight	193	328	114
seed weight	9	16	10
Technological indices			
Index structure of the grape	34.7	39.0	26.1
Index grain	38.0	28.0	40.0
Index composition of grain	4.7	5.3	2.6
Yield index	3.1	2.6	1.6

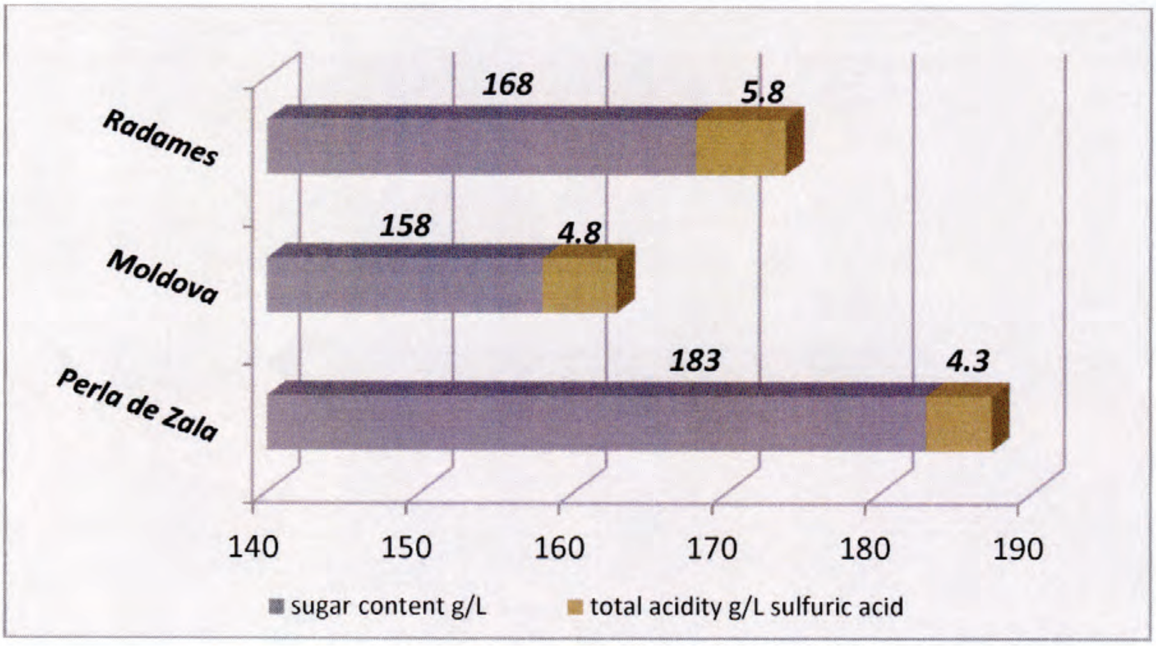

Figure 4. Composition characteristics of grapes.

The GC/MS analysis of volatile compounds revealed a number of compounds that characterize the studied varieties; some of these compounds can be found in higher concentration in comparison with the other identified compounds (Table 4).

Table 4. Volatile compounds from the analyzed musts.

No.	Volatile compounds	***Perla de Zala***	***Moldova***	***Radames***
		ppm		
1	ethyl acetate	2300	2250	3785
2	2-pentanone	1230	1425	1856
3	ethyl butyrate	39	75	144
4	n-propanol	487	119	245
5	ethyl 2-methyl butanoate	122	58	75
6	2-methyl 3-buten-2-ol	54	65	39
7	3-pentanol	85	70	115
8	hexanal	125	89	76
9	(Z)-2-hexenal	258	896	345
10	ethyl hexanoate	75	253	85
11	ethyl caproate	296	387	128
12	1-octene-3-ona	12	-	3
13	methyl hexanoate	25	45	14
14	1-hexanol	1230	985	605
15	ethyl 3-hidroxy butanoate	230	445	120
16	phenylacetaldehyde	2	45	15
17	cis 3-hexen-1-ol	85	64	59
18	ethyl caprylate	758	450	589
19	ethyl 2-hydroxy 4-methyl pentanoate	72	90	28
20	phenyl acetate	75	112	89
21	ethyl-2-hydroxy-3-phenylpropanoate	125	154	115
22	2-phenylethanol	57	81	15
23	caprylic acid	4620	3560	1250
24	delta-3-carene	1.2	1.1	0.3
25	β-myrcene	0.5	0.2	trace
26	limonene	6.8	21.2	8.5
27	p-cymene	1.2	1.4	trace
28	β-pinene	0	0.1	trace

Esters are formed in grape fermentation process in large quantities by enzymatic esterification and in the process of maturation and aging of wine by chemical esterification (VIŞAN, 2015). Thus, regarding esters, there were identified: Ethyl acetate, Ethyl butyrate, Ethyl 2-methyl butanoate, Ethyl hexanoate, Ethyl caproate, Methyl hexanoate, ethyl 3-hidroxy butanoate, Ethyl caprylate, Ethyl 2-hydroxy 4-methyl pentanoate, Phenyl acetate and Ethyl-2-hydroxy-3-phenylpropanoate (Fig. 5).

Among aldehydes, there were identified: Hexanal, (Z)-2-hexenal, phenylacetaldehyde. The higher alcohols were represented by: n-Propanol, 2-methyl 3-buten-2-ol, 3-pentanol, 1-Hexanol, Cis 3-hexen-1-ol and 2-phenylethanol. (Fig. 6). The identified terpenes were, in order of concentration at studied varieties: limonene, p-cymene, delta-3-carene, β-myrcene, β-pinene (Fig. 7).

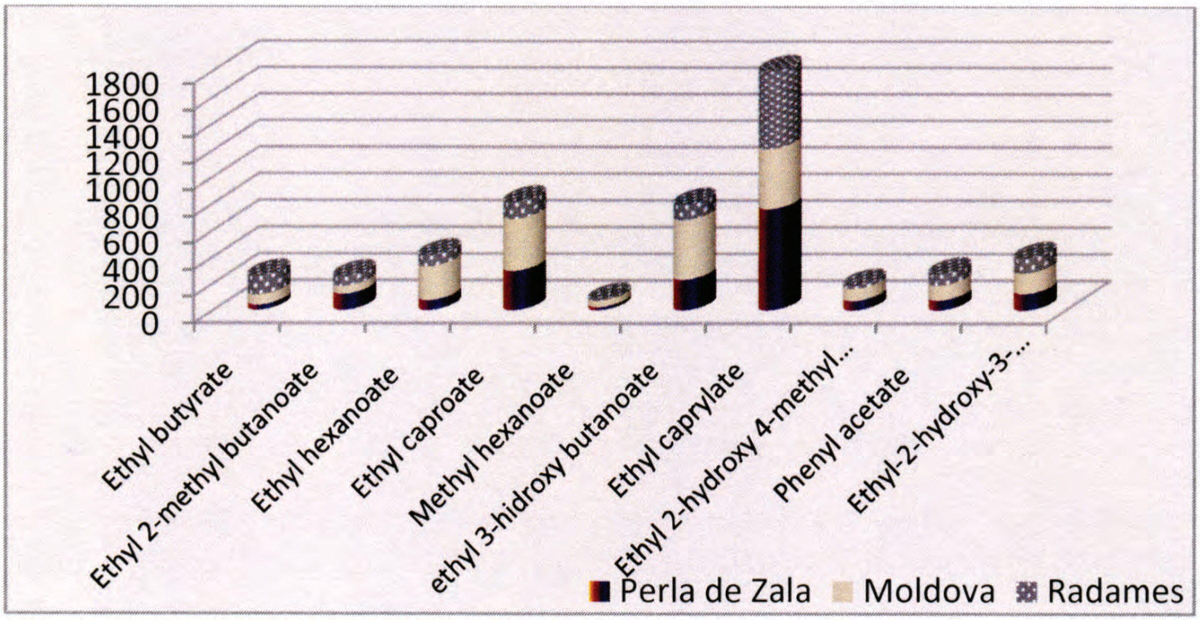

Figure 5. The main esters of analyzed musts, ppm.

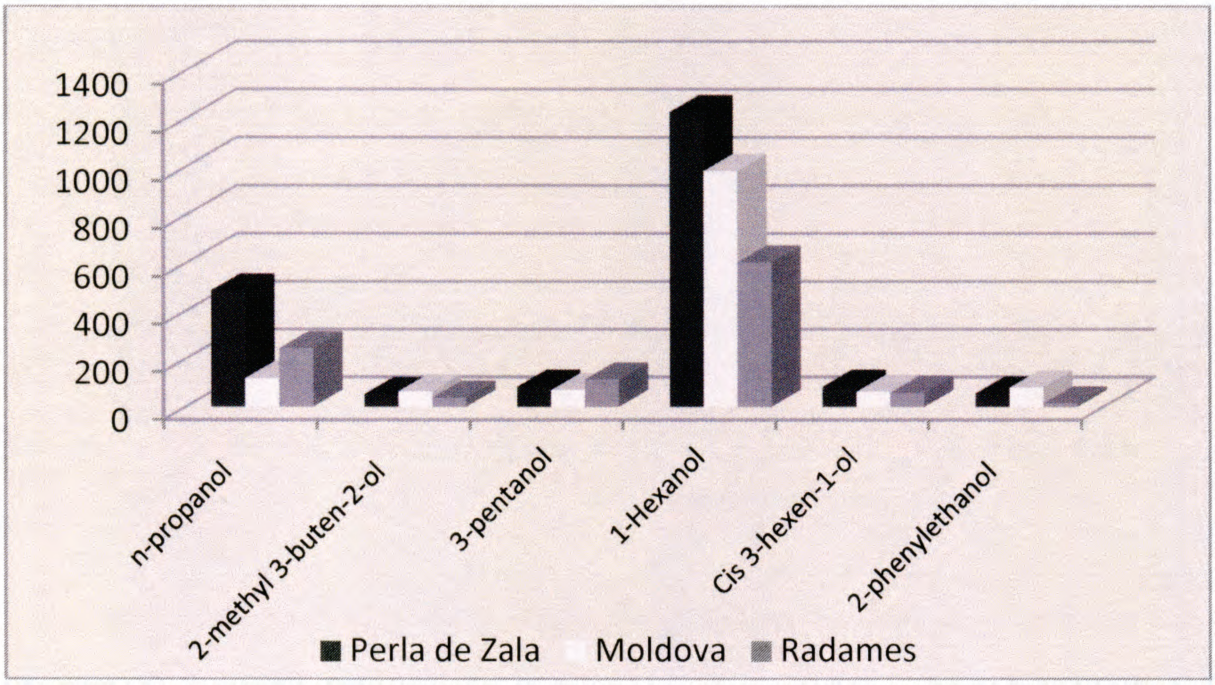

Figure 6. Concentration of higher alcohols, ppm.

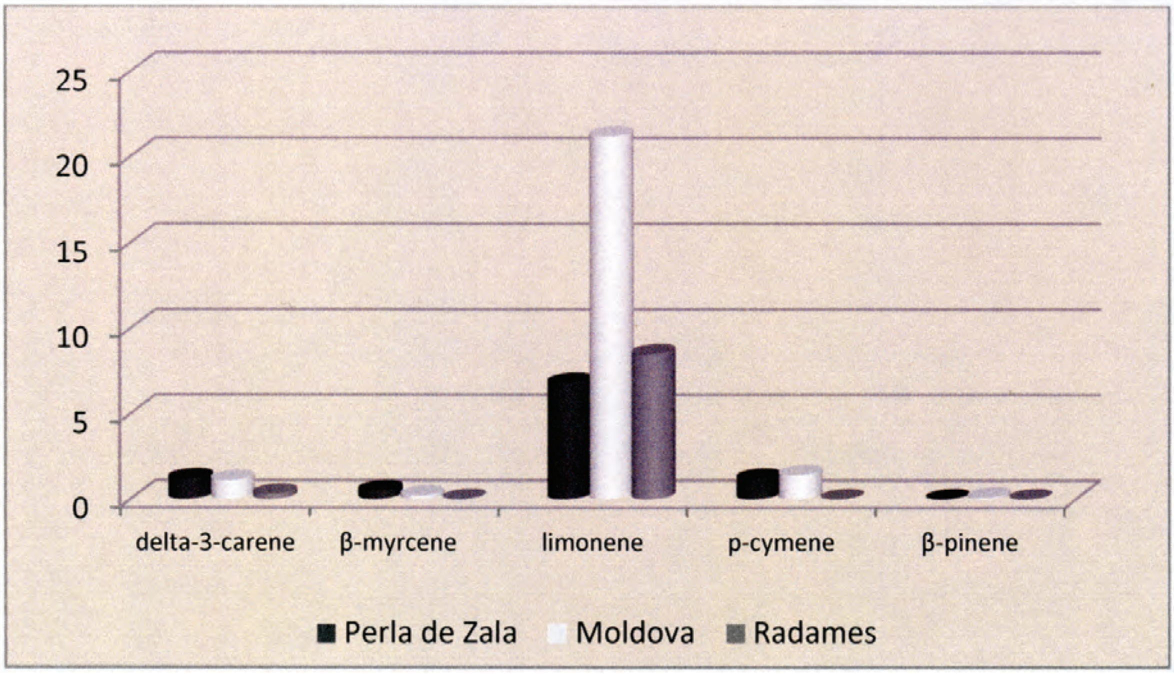

Figure 7. Concentration of terpenes, ppm.

CONCLUSIONS

The beginning of the vegetation period for the vine varieties with biological tolerance analyzed under the conditions of Giurgiu area was advanced by 7-10 days compared to other areas of culture.

The longest vegetation period was recorded by *Radames*, 200 days.

The varieties show a medium fertility, with a percentage of fertile shoots between 60.2% (*Perla de Zala*) and 68.2% (*Radames*).

The highest values of the indices of productivity (absolute and relative) were registered by Moldova variety, the average yield of the variety being 13.8 t / ha.

Perla de Zala variety accumulates a high sugar content of 183 g/L, but records a gluco-acidimetric index of 42, higher than the optimal value. *Moldova* registered the smallest sugar content in grapes (158 g/L).

By GC/MS analysis 28 volatile compounds were identified and dosed: esters, aldehydes, higher alcohols, terpenes, etc.; in high concentrations, there were identified 1-hexanol and n-propanol, ethyl caprylate and ethyl 3-hydroxy butanoates, as well as limonene, terpene in higher concentration as compared to the other identified terpenes.

REFERENCES

BAEK H., CADWALLADER E., MARROQUIN E., SILVA J. 1997. Identification of predominant aroma compounds in *muscadine* grape juice. *Journal Food Science*. **62**: 249-252.

CATTELL H . & STAUFFER H.L. 1979. *The Wines of the East. II. The Vinifera*. L&J, Photojournalism. Lancaster. 125 pp.

ELFVING D.C., DALE A., FISHER K.H., MILES N., TEHRANI G. 1992. *Fruit Cultivars – A Guide to Commercial Growers*. Publication RV-5-92-15M. Ontario. 245 pp.

GALET P. 1979. *A Practical Ampelography – Grapevine Identification*. Ithaca. N.Y. 248 pp.

GRECU V. 2010. *Soiurile rezistente de vita de vie si particularitatile lor de cultura*. Edit. MAST. Bucuresti. 208 pp.

GUTH H. 1997. Identification of character impact odorants of different white wine varieties. *Journal Agricol Food Chemistry*. **45**: 3022-3026.

LE GUEN S., PROST CAROL, DEMAIMAY M. 2000. Critical comparison of three olfactometric methods for the identification of the most potent odorants in cooked mussels. *Journal Science Food Chemistry*. **48**: 1307-1314.

MOLDOVAN S.D., CRISTEA ST., BANCILA AL., POPA I. 1994. *Radames*, soi rezistent pentru vinuri albe. *Analele I.C.V.V*. **14**: 125-127.

OŞLOBEANU M. 1980. *Viticultură generală şi specială*. Edit. Didactică şi Pedagogică, Bucureşti. 667 pp.

SEROT TH., PROUST C., VISAN LUMINITA, BURCEA MIRELA. 2001. Identification of the Main Odor-active Compounds in Musts from French and Romanian Hybrids by Three Olfactometric Methods. *Journal Science Food Agricol*. **49**: 1909-1914.

TARDEA C. & DEJEU L. 1995. *Viticultură*. Edit. Didactică şi Pedagogică. Bucureşti. 504 pp.

TOMOIAGA LILIANA. 2006. *Bolile viței de vie*. Edit. Mediamira. Cluj-Napoca. 351 pp.

TRANCHANT J. 1995. *Manual pratique de chromatographie en phase gazeuse*; IV-eme edition, Edit. Masson. Paris. 700 pp.

VIȘAN LUMINIȚA, POPA O., BĂBEANU NARCISA, TOMA R., SEROT T. 2007. Analytical Methods for Quantitative Identification of Aroma Compounds in Grape Juice of Resistant Varieties. *Lucrări științifice-seria F-Biotehnologii*. **12**: 33-41.

VIŞAN LUMINIȚA., DOBRINOIU RICUȚA, GUIDEA-DĂNĂILĂ SILVANA. 2014. Behavior of wine varieties with biological resistance under the south Romanian conditions. *Scientific Papers, Series B. Horticulture.* Edit. Romanian Academy. Bucharest. **58***:* 229-234.

VIŞAN LUMINIȚA, DOBRINOIU RICUȚA, GUIDEA-DĂNĂILĂ SILVANA. 2015. The Agrobiological Study, Technological and olfactometry of some vine varieties with Biological resistance in southern Romania. *Agriculture and Agricultural Science Procedia*. Elsevier. **6**: 623-630.

VIŞAN LUMINIȚA. 2015. *Biotehnologii fermentative. Biochimia şi Biotehnologia vinului*. Edit. Invel-Multimedia. București. 346 pp.

Vișan Valerica Luminița[1], Tamba-Berehoiu Radiana-Maria[1],
Popa Ciprian Nicolae[2], Dănăilă-Guidea Silvana Mihaela[1]
[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd, zip code 011464, Bucharest, Romania.
Farinsan SA, Grădiștea village, Giurgiu district, Romania.
E-mail: l_visan@yahoo.com

Received: March 31, 2017
Accepted: August 12, 2017