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NEW VARIETIES OF VINE RULES IN RELATION TO THE EUROPEAN UNION THE REQUIREMENTS COMPARED BY BIOCHEMICAL

ALEXANDROV EUGENIU¹

Abstract. The process of obtaining distant hybrids, as well as any crossing of cultivars of V.vinifera L. with representatives of species possessing the necessary qualities (resistance to diseases and pests, low temperature, etc.) may change the spectrum of chemical and biochemical compounds responsible for flavour, colour and taste of grapes, obtained juice and wine. Botanical description of distant hybrids was performed during all phases of the vegetative stages; the organs of the plants were studied from spring, at bud unfolding, until early autumn, at the fall of the leaves. For the determination of diglucoside-3,5-malvidin, the fluorimetric method, for determining the methyl anthranilate, the gas chromatographic method was applied. Based on the analyzes of biochemical constituents of grapes of the distant hybrids: DRX-M4-578; -502; -571; -660; -609; -580; etc., compared to the traditional cultivars "Feteasca albă", "Neteasca neagra", "Rară neagră" and "Negru de Ialoveni", it has been found that these varieties are similar. So, the distant hybrids of grapevine haven't inherited unwanted characters for vines, some of them are strictly limited (diglucoside-3,5-malvidol). These hybrids don't have the specific characteristics of direct production hybrids, characterised by the foxat taste of the grape berries, caused by the presence of the methyl anthranilate. The distant hybrids studied according to the classical uvologic and technological principles can be classified as follows: distant hybrids are attributed to the table vine species (DRX-M4-502; DRX-M4-512 etc.) and distant hybrids have mixed properties (DRX-M3-3-1; DRXM4-580; DRX-M4-640 etc). According to the physical and biochemical indices of the grapes of the studied distant hybrids, their characteristics are similar to European vine species.

Keywords. berry, diglucozide-3,5-malvidol, methl anthranilate, hybrids, resistnce.

INTRODUCTION

The development of culture of the vine throughout of his evolution has known multiple methods of improvement, such as natural selection or directed (intraspecific, interspecific, clonal, genetic engineering etc.). Today it is very necessary to obtain vine varieties productive and qualitative characters, and absolutely resistant to attack by phylloxera (root and leaf).

The process of obtaining hybrids of vines, as well as any cross varieties of *Vitis vinifera* L. with representatives from the donor species of qualities necessary (resistance to diseases and pests winter low temperatures etc.) can bring to the changing spectrum chemical and biochemical compounds responsible for flavor, color and taste of berries, juice and wine obtained.

According to European Union requirements in the production of wine products, the chemical composition of the raw material must meet some strict requirements, for example diglucozid-3,5-malvidol not exceed the limit of 15 mg/dm³.

Recently the World Organisation of Vine and Wine discussed the issue of reducing the index wines at the limit of 5 mg/dm³, which requires to be monitored in the selection of vines to try and approve only varieties with low content of this phenolic - diglucozid-3,5-malvidin [7].

Another important compound juice berries vine distance hybrids of any order, including the is methyl anthranilate (3,4-benzoxazole), which has the main role in the creation of taste and smell (aroma) of frame (naphthalene and / or phenol) [9].

Methyl anthranilate is a chemical compound benzoxazoles group, formed in grapes (especially direct producer hybrids) in amounts of 0,2 to 3,5 mg/dm³ of must (juice). It is found in the wine with the same concentrations of volatile aromatic another chemical compound - ethyl isoamyl [9]. That is why this important chemical compound in the juice of berries new hybrids intraspecific selection requires determined, studied and taken as a criterion for selection [13].

International Organisation of Vine and Wine in 2004 established the methanol concentration limit of 250 ml/l for white wines and 400 ml/l for red wines.

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MATERIAL AND METHODS

In this study were included and distant hybrid of vine *Vitis vinifera* L. x *Muscadinia rotundifolia* Michx., created in the Botanical Garden (Institute) of the ASM, (DRX-M3-3-1; DRX-M4-502; -512; -571; -578; -580; -609; -660). These new strains were tested for resistance to attack the root and the leaf phylloxera, as well as mildium, oidium, botrytis. Berry juice and the wine were tested on content: methyl anthranilate, diglucozide-3,5-malvidin, phenolic substances etc. [1, 2, 3, 11].

The study included ten varieties of vines created of the VCR (Vivai Cooperativi Rauscedo, Italy, Udino), including five varieties of grapes with white berries (Fleurtai (UD-34.111), Soreli (UD-34.113), Early Sauvignon (UD-76.026); Petit Sauvignon (UD-55.098); Sauvignon d´ore (UD-55.100) and five varieties of red berries with juice (Petit Cabernet (UD-58.083); Royal Cabernet (UD-32.078); Royal Merlot (UD-31.125); Petit Merlot (UD-31.122); Julius (UD-36.030) [1].

As compared the traditional varieties were used "Feteasca alba", "Feteasca neagra", "Rara Neagra" and "Negru de Ialoveni".

For the quantitative and qualitative determination of the diglucoside-3,5-malvidin, the quantitative and qualitative fluorimetric method was applied. For determining the methyl anthranilate, the gas chromatographic method was applied [4, 5, 6, 8, 9, 11].

At the biochemical and uvologice analyzes were used methods disclosed in the technical regulations "Analytical methods in the manufacture of wines" [10, 12].

RESULTS AND DISCUSSIONS

In the contemporary agro biological science are the achievements carried in the field of intraspecific selection, beginning with the second half of the XXI century resulted in obtaining new varieties with increased resistance to biotic and abiotic acceptable qualities such as the those in Germany, Hungary, Italy, France, Romania, Moldova, Ukraine, Russia etc.

Appreciating the true value of these achievements agrobiologic characters specific of varieties obtained, it should be noted that the cultivation of these varieties imposes mandatory the grafting protaltoi their North American (resistant to phylloxera), which considerably raises the cost of the planting material production and that of the establishment of a unit - plantation of vines. In addition most of the above mentioned varieties are relatively resistant to the main the contaminants during the growing season (mildium, mildew, botrytis), and lately is observed sensitivity to Agrobacterium tumefacium (cancer bacterial vine) and Flovecence d'or (drying the golden of the arms).

It is judged increased the resistance to low temperatures this winter varieties, which allow them to cultivating on the espaliers and fertile buds diminish loss of in years old with frosty winters. Some of these varieties, however, in some years, are the attacked by phylloxera leaf under the conditions which the harvest matures weak and the immature the young shoots enters into winter.

The investigations were aimed at to check whether by obtaining distant hybrids have not transmitted specific to features direct producer hybrids varieties, of which indexes methyl anthranilate to the concentration of varies from 0,30 mg/dm³ up to 3,6 mg/dm³. The data obtained attest to similarity between the distant hybrids and the classical the varieties: the varieties with the berries green-yellow or green straw distant hybrids, this index attesting 0,08 to 0,17 mg/dm³. These results attest to resemblance of the distant hybrids DRX-M4-571; -578; -609; DRX-M3-3-1 the content of methyl anthranilate with the classic of grapes the variety of "Feteasca alba" of the species *Vitis vinifera* L.

And new varieties of the distant hybrids - DRX-M4-660 and DRX-M3-3-1 with the berries red-violet (pomegranate), the content of that constituent biochemical values from 0,21 to 0,24 mg/dm³ - is attest to similarity to the classic variety "Rara neagra" of the species *Vitis vinifera* L., the fresh juice that has accumulated 0,27 mg/dm³ of methyl anthranilate (tab. 1.).

Table 1. The content of methyl anthranilate, diglucoside-3,5-malvidin and anthocyanins in grapes of distant hybrids of grapevine (Vitis vinifera L. x Muscadinia rotundifolia Michx.)

Form	The content of the compounds, mg/dm ³			
	Methyl anthranilate	Diglucoside-3,5-	Anthocyanins	
		malvidin		
Distant hybrids of grapevine (V.vinifera L. x M.rotundifolia Michx.)				
DRX-M4-502	0,08	-	-	
DRX-M4-580	0,09	-	-	
DRX-M4-512	0,13	-	-	
DRX-M4-578	0,15	-	-	
DRX-M4-609	0,16	-	-	
DRX-M4-571	0,17	-	1	
DRX-M4-660	0,21	7,7	640	
DRX-M3-3-1	DRX-M3-3-1 0,24		513	
Varieties of the Vitis vinifera L.				
Feteasca albă	0,11	-	-	
Feteasca neagra	0,19	7,4	737	
Rară neagră	0,27	4,9	469	
Negru de Ialoveni	0,49	74,0	861	

The organoleptic assessments of the quality of berries to the eight distant hybrids and the classical varieties have allowed us to note the absence of the flavoring (of smell) and taste typical of "foxat" direct producer hybrids, but especially of the North American varieties of *Vitis labrusca* (Concord, Isabella, Noah, Lidia, Delaware, Othello etc.).

Only the "Negru de Ialoveni", intraspecific hybrid was determined by the same method gaseous phase chromatography, an increased concentration of methyl anthranilate 0,49 mg/dm³, what reaches values close to direct producer hybrids [7].

Another important characteristic of the hybrids of any origin is the concentration of diglucozid-3,5-malvidol, which is strictly limited to the export wine to the European Union to the limit of ≤ 15 mg/dm³. In this order of ideas, it went on the determination of this component anthocyanins (in berry colorant) as the method widely known and used in the European Union.

The results obtained demonstrates that this index in the fresh juice of distant hybrids varies between 7,7 mg/dm³ and 9,3 mg/dm³ of diglucozid-3,5-malvidol (DRX-M4-660; DRX-M3-3-1) and the classical variety of "Rara neagra" ("Cricova", c. Lucesti, r. Cahul) it is found just 4,9 mg/dm³ of diglucozid-3,5-malvidol. These results allow us to state that after the index values the concentration of diglucozid-3,5-malvidol, F4 distant hybrids of varieties obtained from Botanical Garden (Institute) of the ASM do not differ practically of the classic variety "Rara neagra".

Exception in the investigations carried out, make the intraspecific hybrid "Negru de Ialoveni" with 74,0 mg/dm³ of diglucozid-3,5-malvidol this variety of berries juice of vines collection of Practical Scientific Institute of Horticulture and Food Technology.

After the content of the phenolic compounds, including antocianelor, berries from the juice direct hybrids in comparison to that of the classic varieties *Vitis vinifera* L. (Feteasca alba and Rara neagra), these varieties not radically different, but just within the limits of 184-260 mg/dm³ phenolic compounds in green-yellow berries (straw) DRX-M4-571; DRX-M4-512 etc. and 1987-2316 mg/dm³ of distant hybrids DRX-M3-3-1 and DRX-M4-660.

And the index of the anthocyanins concentration in stained the varieties does not attest to fundamental difference between the distant hybrids and the classic varieties of the Rara neagra: from 513 $\,\mathrm{mg/dm^3}$ to 640 $\,\mathrm{mg/dm^3}$ for the distance hybrids and a content (regular) of the 469 $\,\mathrm{mg/dm^3}$ the Rara neagra from the group of *Vitis vinifera* L.

The juice of berries of the variety of vines "Negru de Ialoveni" contains high quantities of phenolic compounds (about 2790 mg/dm³) and anthocyanins (861 mg/dm³).

The organoleptic appreciation of the fresh juice obtained from the distant hybrids created at the Botanical Garden (Institute) of the ASM in comparison with the berries juice of the classic varieties "Feteasca alba" and "Rara neagra" from "Cricova" S.A. made during the harvest of 2014 [the appreciation the flavoring (of smell) and taste etc.] have enabled us to certify a likeness of berries color, of the flavoring and taste the juice extracted from them, with small nuances at every variety under investigation.

Table 2.

Acidity

Hybrids	titratable acidity, g/dm ³	pН	tartaric acid,	malic acid, g/dm ³
			g/dm³	
DRX-M ₃ -3-1	5,5	3,6	4,7	2,9
DRX-M ₄ -502	6,0	3,5	3,7	2,4
DRX-M ₄ -512	6,8	3,1	4,1	3,1
DRX-M ₄ -571	6,6	2,9	4,1	2,7
DRX-M ₄ -578	6,1	3,3	3,2	2,1
DRX-M ₄ -580	6,2	3,1	4,3	2,4
DRX-M4-609	5,9	3,4	3,7	1,9
DRX-M ₄ -640	6,4	3,0	4,5	2,9

At general the score for have been highlighted gustatory-olfactory the hybrids with green-yellow berries: DRX-M4-609 with 9.5 points and DRX-M4-502 with 9.0 points. Among the hybrids with red-purple berries (pomegranate) was highlighted the hybrid DRX-M3-3-1 with 9.3 points.

Evident, the representatives of the species *Vitis vinifera* L. (Feteasca alba, Rara neagra) were gained 9,9 points, respectively, 9,8 points, which shall be entered in their traditional values and typical.

The intraspecific variety of "Negru de Ialoveni" the score for did not exceed 8.9 points, too tannic taste and practically neutral in flavoring.

Table 3.

Sugars

Hybrids	Sugar, g/dm ³	Glucose, g/dm ³	Fructose, g/dm ³
DRX-M ₃ -3-1	166	84,2	80,5
DRX-M ₄ -502	163	85,5	74,1
DRX-M ₄ -512	159	82,0	70,3
DRX-M ₄ -571	144	77,1	65,8
DRX-M ₄ -578	158	81,3	69,7
DRX-M4-580	167	84,8	80,7
DRX-M4-609	163	83,7	78,3
DRX-M ₄ -640	151	78,8	70,5

As for the physico-chemical and biochemical indexes after the concentration of organic acids (tartaric and malic acid), for all hybrids distances can be attested at European of vines species *Vitis vinifera* L., tartaric acid ranging within the limits of 3.2 g/dm³ to 4.7 g/dm³, and malic acid: from 1.9 g/dm³ to 3.1 g/dm³ (tab.2.).

The concentration of the glucose and fructose in the medium constitutes 95.5 to 99.3% of the total concentration of the sugars (100%).

The report of the glucose/fructose it is typical of the European vines varieties and ranges from 1.04 up to 1.17 (tab. 3.).

The concentration of the phenolic substances demonstrates that distant hybrids meet the criteria of vines variety rights for consumption.

The amount of these substances of distance hybrids of the vine with the green-yellow berries varies between $201-293~\text{mg/dm}^3$ and hybrids with berries blue-violet nuance - from the $777~\text{mg/dm}^3$ to $809~\text{mg/dm}^3$.

It is gratifying that the concentration of resveratrol, as biological compound for human nutrition, it is relatively increased in comparison with the classic of vines the varieties (4,1 to 5,3 mg/dm³), and distant hybrids varies in boundaries of 5,7 mg/dm³ to 11,7 mg/dm³ (tab. 4.).

Chemical properties

Table 4.

Hybrid	Phenolic substances, mg/dm ³	Resveratrol, mg/dm ³	Pectins, mg/dm ³
DRX-M ₃ -3-1	809	8,5	714
DRX-M ₄ -502	292	8,1	580
DRX-M ₄ -512	288	6,6	517
DRX-M ₄ -571	263	5,7	703
DRX-M4-578	274	7,3	647
DRX-M4-580	293	6,8	439
DRX-M ₄ -609	201	7,7	516
DRX-M ₄ -640	777	11,7	697

According to the physical and biochemical the indices berries distant hybrids studied their characteristics are similar to the european of vines variety rights: total nitrogen varies in the range of 563 mg/dm³ up to 740 mg/dm³, phosphorus - 179-263 mg/dm³, calcium - 107-156 mg/dm³, potassium - 1367-2013 mg/dm³, magnesium - 103-144 mg/dm³ (tab. 5.).

Chemical properties

Tabelul 5.

Hybrid	$N, mg/dm^3$	P , mg/dm^3	$Ca, mg/dm^3$	K , mg/dm^3	$Mg, mg/dm^3$
DRX-M ₃ -3-1	563	263	156	2013	109
DRX-M ₄ -502	590	199	117	1710	123
DRX-M ₄ -512	591	240	109	1907	151
DRX-M ₄ -571	621	213	121	1415	103
DRX-M ₄ -578	640	240	131	1800	110
DRX-M ₄ -580	611	179	127	1881	144
DRX-M4-609	583	186	141	1919	119
DRX-M ₄ -640	740	223	107	1367	127

International Organization of Vine and Wine in 2004 has established of the methanol the concentration limit of the 250 ml/l for white wines and 400 ml/l for red wines (Directive 19/2004 OIVV).

In order not to mix the red grapes of vines *Vitis vinifera* L. direct producer hybrids in the European practice has started to the establishment of a limit in component - test, which is a blue-violet anthocyan of the old chemical name malvidina (Malvinas), and the new - diglucozid-3,5-malvidol. He is a natural component innocuous, clean and not possesses harmful properties. (fig.1.).

According to the European Union requirements the concentration of the diglucozid-3,5-malvidol juice of the berry do not have to exceed the limit of 15 mg/l. (fig. 2.).

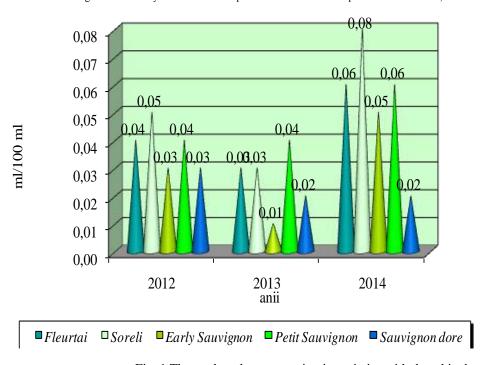


Fig. 1 The methanol concentration in varieties with the white berry.

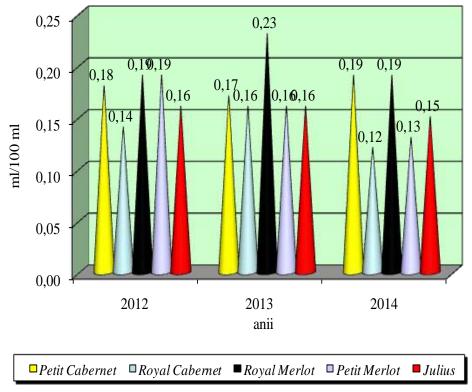


Fig. 2 The methanol concentration in varieties with the red berry.

From the results presented in Fig. 3 found that the only in the new variety Petit Cabernet of the diglucozid-3,5-malvidol concentration does not exceed the limits set of 15 mg/l and entered the in the cluster in such a way varieties of *Vitis vinifera* L.

As regards the varieties of Julius, Petit Cabernet, Merlot Petit Royal Merlot, Cabernet Royal attesting to a high concentration of this anthocyan innocuous and harmless, but which may currently just create impediments to their approval in some countries European Union.

Nutritionists and dietology says that the human body in its evolution has created sufficient enzymatic systems recovery, metabolism of anthocyanins and other compounds derived turning them into necessary and useful

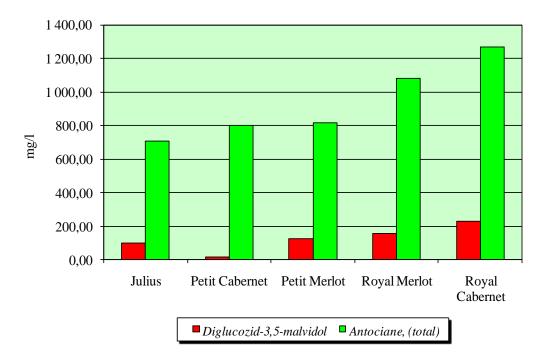


Fig. 3 The concentration of diglucozid-3,5-malvidol the total amount of anthocyanins.

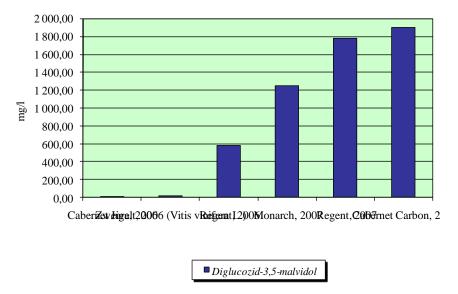


Fig. 4 The concentration of diglucozid-3,5-malvidol of the juice berries of vines varieties (red) created in Germany.

Interesting results from the view point uvologic and oenological have been obtained by the specia of new varieties of vines from Germany, which proves that us can be get the character the selection agrobiologic intraspecific important (resistance, productivity and quality), but with the a high content in diglucozid-3,5-malvidol (580 mg / l in the wine variety Regent and 1,900 mg / l in wine Cabernet Carbon).

Interesting results from the view point uvologic and oenological have been obtained from of vines the varieties created in Germany, proves that which we can be get the important agrobiologic character of the selection intraspecific (resistance, productivity and quality), but with the a high content in diglucozid-3,5-malvidol (580 mg/l in the wine variety Regent, and 1900 mg/l Carbon Cabernet wine).

Of course in comparison with the varieties of *Vitis vinifera* L., Cabernet (Jura, France) and Zweigelt (Rhein, Germany) investigated the index not exceeding the limits 15 mg/l, established by the specialized international organizations (fig. 4).

CONCLUSIONS

- 1. The creation of varieties of vines resistant to phylloxera root and foliar in mildium powdery mildew, gray mold and other biotic and a high resistance to low temperatures in winter and drought, will enable truly solving the problem of producing table grapes and for industrial processing biological (ecological). Due to their high agro biological properties, these varieties treated during the season moderate and limited (strictly in accordance with European legislation "bio") will allow to ensure high hygiene and berries and grapes curativitate.
- 2. The new varieties must have the a period precocious maturing of the grapes to ensure the planting and nurturing their at the frontiers of northern of viticulture various continents, ensuring at the same time and maturation chord plants for the wintering success, allowing their cultivation on the espalier with medium and high stem to the maximum possible mechanized and automated cutting processes agro- technical bound, sprinkled, manually or mechanically with combine harvesting etc.
- 3. It is desirable that the grapes varieties of grapes for current consumption to possess high transportability order to be exported at great distances, and the technical capacity low absorption of oxygen in the air and protection of berries, juice and wine at oxidation (redox processes reductive low).

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