

Ampelometric, Genetic and Sanitary Evaluation of CV. Vranac

Vesna MARAS¹⁾, Marina BOGICEVIC²⁾, Milena TOMIC¹⁾, Vesna KODZULOVIC¹⁾,
Sanja SUCUR¹⁾, Miroslav CIZMOVIC³⁾, Danijela RAICEVIC³⁾

¹⁾”13Jul Plantaze”, Department of Development, Put Radomira Ivanovica num.2, 20000 Podgorica, Montenegro; vesnam@t-com.me

²⁾Department of Crop Production, Faculty of Agriculture, University of Milan, Via Celoria num.2, 20133 Milano, Italy; marina.bogicevic@unimi.it

³⁾ Biotechnical faculty, Department for Viticulture and Enology, University of Montenegro, Mihaila Lalica num.1, 20000 Podgorica, Montenegro; miroslaw@t-com.me

Abstract. Vranac is autochthonous grape variety of Montenegro, being of significant importance for Montenegrin economy. Grape of this variety has been used for the production of red wine of top quality, very appreciated at the domestic and foreign market. Researching was done during three years in three locations in sub-region Podgorica. Beside ampelometric and uvologic indicators for vranac variety, average sugar content and total acids in must (22.08% and 4.95 g/l). Based on three years of investigation, an average largest number of bunches is noticed in population of vranac variety in location Nikolj crkva and the largest weight of bunch is measured in location Cijevna. The largest yield of grape per vine is reached in location Nikolj crkva. The content of total anthocyanins is medium high while the value of total flavonoids is high. Total polyphenols, proanthocyanidols and flavans which react with vanilla reached medium values in years of researching. Using Elisa test, samples were tested for viruses: Arabis Mosaic virus (Ar); Fanleaf (FL); inf.mix (F+GVA+Lr3); Fleck virus (K), inf.mix (GVA+Lr3); Leafroll virus type1 (LR1); Leafroll virus type3 (LR3); inf.mix Lr1 + Lr3 (Lr1+Lr3). Turned out that of 145 tested samples 17 genotypes (11.72 %) are negative in virus testing. After Elisa test, these 17 samples were analyzed by PCR and indexing after which 4 genotypes (23.53 %) passed complete sanitary control and entered the individual clone selection procedure. The content of total anthocyanins is medium-high, while the value of total flavonoids is high. Total polyphenols, proanthocyanidol and flavans which react with vanilla have reached medium values in examined years.

Keywords: variety, vranac, ampelometric, sanitary status, clone selection

INTRODUCTION

Vranac is Montenegrin autochthonous grape variety, belongs to ecological-geographical group *Proles pontica* (*Convarietas pontica*) and it was described by many authors: Viala and Vermorel, 1910, Ulićević, 1966, Nastev, 1967, Burić, 1995, Cindrić, 2000, Božinovik, 2006, Milosavljević, 2008. DNA analyses (Callo *et al.*, 2008) showed that vranac is closely related to kratosija and that there is probably first-degree relationship (the closest relationship) between these two varieties. In the same paper, based on DNA analyses the author confirms that kratosija has the same DNA profil as zinfandel has, and that relation between vranac and kratosija (zinfandel) as parent-offspring relationship should be confirmed by addition molecular analyses. According to many literature data (Ulićević, 1966, Pejović, 1988, Maraš, 2000) kratosija is autochthonous grape variety that occurred earlier and was introduced in culture of breeding quite earlier before vranac was, also it has very heterogeneous population with more biotypes which are not sufficiently studied. Unlike

kratosija, morphological features of vranac variety are stable. Vranac is expanded in recent years and it became main variety for producing red wines not only in Montenegro, but also in Bosnia and Herzegovina, Macedonia and Dalmatia (Croatia).

The vine of vranac variety is vigorous, flower is hermaphrodite, berry is medium large to large, oblong shape, thin scarfskin, blue color and abundant with blue bloom. Bunch is medium large and large, cilindric, medium dense, rarely loose. Vranac variety has high yield and grape ripen in third epoch (late variety). For reaching a good quality yield it should be cultivated on sunny position and on warm, loose – gravelly, permeable and moderately fertile soil.

Produced wine of grapes of this variety is pleasant, harmonious, specific varietal aroma and taste with percentage of alcohol 11-14 vol% and 5-6 g/l of total acids. It is recognized by intensive color.

Research was done in aim of better informing about population of vranac variety. In paper are shown ampelometric data of population of vranac variety in three years, as well as economic and technological characteristics on three locations of sub-region Podgorica. Sanitary status of population in multi-year period is shown, too. The content of polyphenols was also observed, from beginning of veraison to the ripening of grapes in location Nikolj crkva during 2007-2008 period.

MATERIAL AND METHODS

Research was done in the vineyards in location Nikolj crkva (the distance of planting in vineyard is 2.6x1.2m; 3205 vines/ha), Cijevna (the distance of planting is 2.2x0.7m; 5495 vines/ha) and Dinos (the distance of planting is 2.2x0.7m; 5495 vines/ha)

In location Nikolj crkva vines were formed in the shape of double horizontal cordon, height 70 cm from the ground and in the other two locations (Cijevna and Dinos) vines were formed in the shape of single cordon. Back was constructed by a combination of concrete posts and wire. The mixed pruning was applied.

Data were collected by standard methods for this kind of investigations. Ampelometric measurements and uvological analysis were done during the harvest. Average minimum and maximum values of width and length of bunch, width and length of berry, % peduncle, % scarfskin, % seeds, % flesh and content of sugar and acids in must are shown. Data processing for characteristics: number of bunches, weight of bunch and yield of grape per vine was done by analysis of variance for two-factorial experiment. Significance of differences was determined by LSD test.

The content of polyphenols in scarfskin is observed from beginning of veraison to the full ripening (harvest) in two years of researching in location Nikolj crkva. Based on the methods (Di Stefano and Cravero, 1991) the next parameters are determined: total polyphenols, total anthocyanins, total flavonoids, proanthocyanidols and flavans which react with vanilla. Sampling was done on 20 vines and on berries (400) which are picked randomly from different part of bunch. Then the scarfskin of 100 berries was removed and placed in the buffer with pH 3.2 (Di Stefano and Cravero, 1991), after which it was frozen on - 29°C 'till the moment of analytical tests.

Sanitary status of population is analyzed by Elisa test, PCR and by indexing. In multi-year period 145 vines were tested.

RESULTS AND DISCUSSION

Ampelometric and uvological variability – Tab. 1 shows ampelometric data for population of Vranac variety: length and width of the bunch and the berry, and also the part of mechanical composition of the bunch and the berry (uvological analysis). The Tab. 1 also shows an average value of sugar content and total acids in must of vranac variety during three years researching on three different locations (Nikolj crkva, Cijevna and Dinos).

Tab. 1.

Ampelometric data of population of Vranac variety (2008/2010)

Location	Bunch length (cm)	Bunch width (cm)	Berry length (mm)	Berry width (mm)	Peduncle (%)	Scarfskin (%)	Seeds (%)	Flesh (%)	Sugar (%)	Total acids (g/l)
Nikolj crkva	17,33	9,67	19,50	15,47	2,30	9,61	3,48	84,61	21,77	5,09
Cijevna	15,25	9,25	16,30	14,70	2,13	14,45	3,85	79,58	22,97	4,48
Dinos	14,70	8,43	14,03	13,07	2,41	18,37	2,54	76,68	21,50	5,28
X	15,76	9,12	16,61	14,41	2,28	14,14	3,29	80,29	22,08	4,95
Min.	14,70	8,43	14,03	13,07	2,13	9,61	2,54	76,68	21,50	4,48
Max.	17,33	9,67	19,50	15,47	2,41	18,37	3,85	84,61	22,97	5,28

An average length and width of bunch of population of vranac variety during three years of researching on three different locations in sub-region Podgorica was 15.76x9.12 cm. Berry's length and width was 16.61x14.41 mm. An average peduncle percentage was between 2.13 to 2.41 %. In determining of bunch's and berry's mechanical composition of vranac variety i.e. of bunch's structure, an average scarfskin percentage was 14.14 %, seeds 3.29 % and flesh 80.29 %. Sugar content in must varied from 21.50% to 22.97 %, while total acids varied between 4.48 g/l to 5.28 g/l.

Number of bunches per vine – this is one of the crop indicators of variety which directly influence on yield of grape. The largest number of bunches per vine in population of Vranac variety is noticed in the location Nikolj crkva, statistically significantly larger in comparison to another two locations.

Tab. 2.

Number of bunches per vine

Location (A)	Year (B)			X (Average)
	2008.	2009.	2010.	\bar{X}_A
Nikolj crkva	18.10 ab	22.40 a	21.00 a	20.50 a
Cijevna	8.50 c	10.70 bc	12.60 bc	10.60 b
Dinos	8.40 c	17.10 ab	16.60 ab	14.03 b
\bar{X}_B	11.67 b	16.73 a	16.73 a	15.04
	LSD _{0.05} = 3.27 LSD _{0.05} = 5.67	LSD _{0.01} = 4.34 LSD _{0.01} = 7.52		LSD _{0.05} = 3.27 LSD _{0.01} = 4.34

Population of Vranac variety in all three examined locations, observing the results by years, reached the least number of bunches per vine in 2008, statistically significantly lower than in 2009 and 2010.

The weight of the bunch – this is biological feature of every variety. The largest weight of the bunch is measured in Vranac variety in location Cijevna, statistically significant larger in comparison to reached weight in location Dinos.

Tab. 3.

Weight of the bunch (g)

Location (A)	Year(B)			X (Average)
	2008.	2009	2010.	\bar{X}_A
Nikolj crkva	260.07 ab	232.72 bc	189.33 c	227.37 ab
Cijevna	191.38 c	213.66 bc	177.78 c	245.71 a
Dinos	318.98 a	191.99 c	226.15 bc	194.27 b
\bar{X}_B	256.81 a	212.79 ab	197.75 b	222.45
	LSD _{0.05} = 34.120 LSD _{0.05} = 59.09		LSD _{0.01} = 45.27 LSD _{0.01} = 78.35	LSD _{0.05} = 34.12 LSD _{0.01} = 45.23

For all three examined locations an average largest weight of bunch was measured in 2008 (256.81 g), statistically significant larger then in 2010. The minimum weight of bunch was measured in 2008 on location Cijevna, which 'caused interaction of factors (AxB) because the largest weight of bunch was reached in the other two examined locations in the same year (2008).

Yield of grape per vine – The largest yield of grape per vine in population of vranac variety was reached in location Nikolj crkva.

Tab. 4.

Yield of grape per vine (kg)

Location(A)	Year(B)			X (Average)
	2008.	2009.	2010.	\bar{X}_A
Nikolj crkva	4.62 ab	5.29 a	3.95 abc	4.62 a
Cijevna	1.58 e	2.22 cde	2.15 de	1.98 c
Dinos	2.63 cde	3.31 bcde	3.63 abcd	3.19 b
\bar{X}_B	2.94 a	3.61 a	3.24 a	3.26
	LSD _{0.05} = 1.34 LSD _{0.05} = 0.77		LSD _{0.01} = 1.78 LSD _{0.01} = 1.03	LSD _{0.05} = 0.77 LSD _{0.01} = 1.03

Yield per vine is statistically significant higher in location Nikolj crkva compared to another two locations. The lowest yield of grape per vine was measured in population of vranac variety in location Cijevna, significantly lower than in location Dinos.

Appeared differences in average, between years of researching in examined locations have not shown statistically significant. An average largest yield per vine in all three examined locations was in 2009 year and the least was in 2008.

Polyphenol ripening - Polyphenol ripening, the content of polyphenols in scarfskin of berry of variety Vranac have been studied from the phonological phase of beginning of berry's coloring (veraison) to the phase of full ripening in location Nikolj crkva in 2007 and 2008 year. In table 5 are shown values of examined parameters with dates of taking samples.

Tab. 5.

The content of polyphenols in the berry's scarfskin of Vranac variety (mg/kg of grape)

	2007					2008			
DATE	8/8	13/8	17/8	23/8	31/8	31/7	14/8	21/8	28/8
Total anthocyanins	582±4,1	1023±13,9	1138±2,8	1309±9,9	1638±1,8	4±1,1	869±1,8	1290±25,1	1339±40,6
Total flavonoids	1990±10,8	2517±56,3	2838±12,9	3288±44,2	3915±36,5	2608±8,1	2027±6,6	2548±50,5	2571±80,1
Total polyphenols	1191±112,9	1259±190,7	1468±144,9	1393±14,4	1667±198,1	1206±97,7	1230±8,9	1340±5,1	1405±68,6
proanthocyanidols	1206±27,1	970±31,2	1072±54,2	1193±110,3	1634±74,7	2524±46,3	1526±77,6	987±45,3	1043±48,2
Vanilla *	526±109,9	389±143,1	454±139,2	447±163,7	615±171,1	1788±25,7	630±10,3	386±27,4	352±1,3
V/P **	0,44	0,4	0,42	0,37	0,37	0,71	0,41	0,39	0,34

An average values of two biological repeating:

*Flavans which react with vanilla;

**Ratio of flavans which react with vanilla and proanthocyanidols

The values of total anthocyanins are medium-high and show increase tendency, with the fact that in 2007 is noticed larger content of total anthocyanins, compared to 2008. It can be explained by favorable climatic conditions which contributed better anthocyanin accumulation. The values of total flavonoids are high, partly due to absorption of certain amount of anthocyanins on the same wave length. The first year of examination has showed steady increase of these compounds, while in the second year that value was almost unchanged.

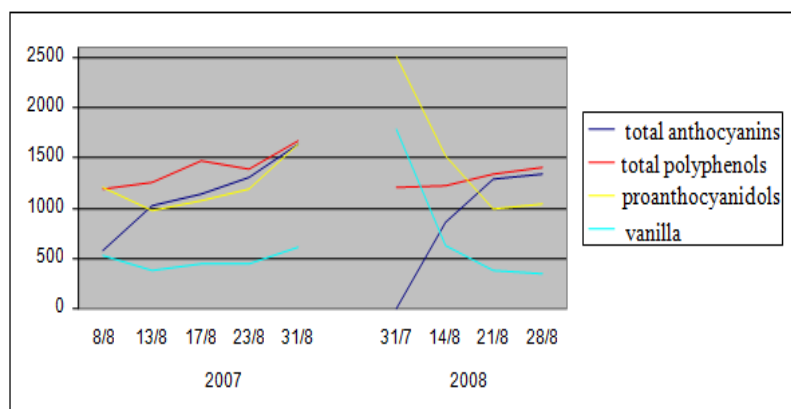


Fig.1. Polyphenol's evolution in berry's scarfskin of Vranac variety

Total polyphenols, proanthocyanidols and flavans which react with vanilla were reached medium values in years of researching and as we can see in the graph 3 movement of values is not uniform. The conditions for biosynthesis of these compounds were favorable in 2007 and their accumulation can vary depending on the climatic conditions, soil, environment and growing techniques in vineyard. The aim is reaching as much content of compounds which are necessary for better maturation of wine and greater color stability of wine (co-pigmentation with anthocyanins).

Ratio V/P reached values which are significantly lower than 1.00, confirming by that from phenological phase of beginning of berry's coloring (veraison) there are mostly

polymerized flavans instead of monomerized in scarfskin of berry.

Sanitary status - Samples of the population of vranac variety were tested on the presence of viruses by Elisa test and PC. It was tested 145 samples (vines). Most samples – 55 vines (37.93%) were infected by virus LR3 and 45 vines (31.03 %) by GVA+LR3. There were not infected vines by AR3 virus. Using Elisa test, the presence of viruses was not identified in 17 vines (11.72%) (Fig. 2).

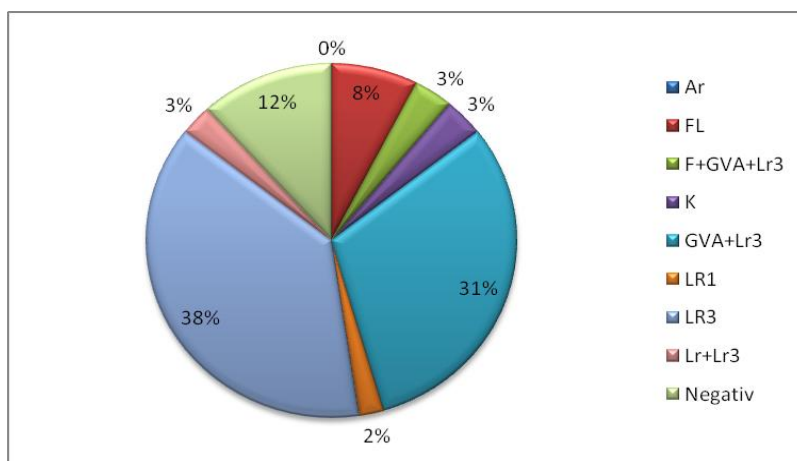


Fig. 2. Infection level of genotypes of cv Vranac with different viruses (Elisa test)

Arabis Mosaic virus (Ar); Fanleaf (FL); inf.mix (F+GVA+Lr3); Fleck virus (K), inf.mix (GVA+Lr3); Leafroll virus type1 (LR1); Leafroll virus type3 (LR3); inf.mix Lr1 + Lr3 (Lr1+Lr3)

Vines which passed sanitary control by Elisa test were also tested by PCR. Of 17 tested vines, 5 vines (29.41%) were infected with RSP and the same (29.41%) with RSP+NN.

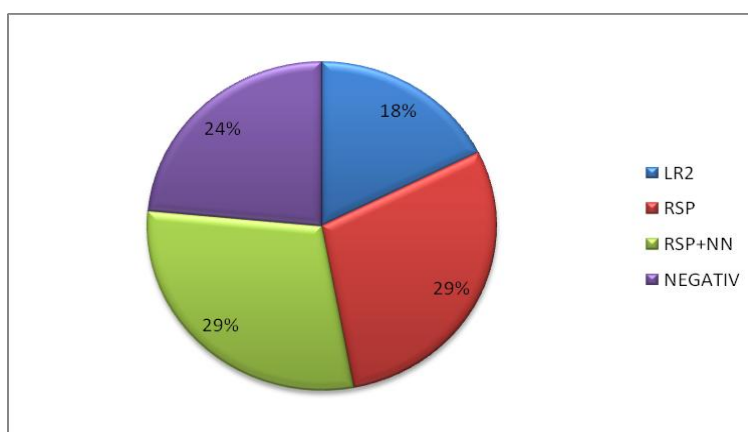


Fig. 3. Infection level of genotypes of cv Vranac with different viruses (PCR and index)

PCR Leafroll virus type2 (LR2), Stem pitting virus (RSP); Stem pitting virus + Vein Necrosis (RSP+NN)

Of total number of tested samples (17), 4 samples passed PCR and indexing (23.53%) – there were no viruses. They represent very useful material which entered in procedure of individual clone selection.



Fig 4. Potential clone 1



Fig 5. Potential clone 2



Fig 6. Potential clone 3



Fig 7. Potential clone 4

CONCLUSIONS

Based on three years results in three examined locations (Nikolj crkva, Cijevna and Dinos) of population of Vranac variety can be concluded:

- The length and the width of the bunch of vranac variety are 15.76x9.12 cm, while the length and the width of berry are 16.61x14.41 mm.

- By analyzing of mechanical composition of bunch and berry it is determined that percentage of peduncle was 2.28 %, seeds 3.29 % and flesh 80.28 %. Beside ampelometric and uvological indicators of Vranac variety it is also shown sugar content and total acids in must (22.08 % and 4.95 g/l).

- Based on three years results, the largest number of bunches per vine was noticed in population of Vranac variety in location Nikolj crkva, while the largest weight of bunch is measured in location Cijevna. The largest yield of grape per vine was reached in location Nikolj crkva.

- Of the 145 tested samples 17 genotypes (11.72 %) were negative on virus testing. After PCR analysis and indexing, 4 genotypes (23.53 %) passed complete sanitary control and entered the procedure of individual clone selection.

REFERENCES

1. Božinovik, Z. (1996). Ampelografija. Agencija "Akademik"-Skopje.
2. Burić, D. (1995). Savremeno vinogradarstvo."Nolit".Beograd.
3. Calo, A., A. Costacurta, V. Maras, S. Meneghetti and M. Crespan (2008). Molecular Correlation of Zinfandel (Primitivo) with Austrian, Croatian, and Hungarian Cultivars and Kratosija, an Additional Synonym, Am. Journal Enol.Vitic., 59:2.
4. Cindric, P., V. Kovač and N. Korać (2000). Sorte Vinove loze. Poljoprivredni

fakultet:Prometej. Novi Sad.

5. Di Stefano, R. and M.C. Cravero (1991). Metodi per lo studio dei polifenoli dell uva. Riv.Vitic.Enol., (2), 37-45.

6. Maraš, Vesna (2000). Ampelografske karakteristike varijeteta sorte vinove loze. Doktorska disertacija. Poljoprivredni fakultet. Zemun-Beograd.

7. Milosavljević, M. (2008). Biotehnika vinove loze, Institut za istraživanja u poljoprivredi „Srbija“, Beograd; „Draganić“, Zemun.

8. Nastev, D. (1967). Specijalno lozarstvo. Univerzitet vo Skopje. Izdanie na Univerzitetot voSkopje. Skopje.

9. Pejović, Lj. (1988). Ampelografska proučavanja varijeteta kratošije. Jugoslovensko vinogradarstvo i vinarstvo, br.3-4. Beograd.

10.Ulićević, M. (1966). Prilog proučavanju osobina najvažnijih sorata vinove loze gajenih u SR Crnoj Gori. Arhiv za poljoprivredne nauke, god X, sv.23 1-10.

11.Viala, P. and V. Vermorel (1910). Ampelographie I-IV. Massonet C^{ie},Paris.