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Research of origin and work on clonal selection of Montenigrin grapevine varieties cv. vranac and cv. kratosija

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Summary

Viticulture production and winemaking of Montenegro are mostly based on growing of autochthonous grapevine varieties. Besides Vranac variety, Kratosija variety takes a significant place in Montenegrin autochthonous grapevine varieties. According to many literature data, Kratosija is an autochthonous grapevine variety whose origin and cultivation started earlier than with Vranac variety. Because of its heterogeneity, it is not so much represented in Montenegrin vineyards and it is mostly found in combination with Vranac variety. The research results about the origin of Vranac variety are shown in this paper. Furthermore, genetic identification which approved originality of Vranac variety is shown and it has also been approved that autochthonous variety Kratosija has the same genetic profile as cv. Zinfandel. The paper presents multi-annual results of examination of variability of their populations and work on clonal selection. Mother vines were selected and vineyards of pre-base and base category of Vranac variety potential clones were planted. The results achieved, in view of manifesting agro-biological, economic and technological characteristics, point to the need for further work on clonal selecton of these grapevine varieties.

Key words: origin, population variability, vranac, kratosija, clonal selection

Introduction

Vranac i Kratosija (Zinfandel) are the most important grapevine varieties for production of red wines in Montenegro. Vranac wine became a national brand as well as the most recognizable and the best product of the "13 jul Plantaze" Company. A group of experts of Milan's weekly magazine "Il mondo" ranked Vranac wine among the 100 best red wines of Europe in 1991.

Kratosija is a Montenegrin autochthonous variety for production of red wines, grown for centuries in Montenegro, which falls into the category of

recommended varieties for quality wine. According to many literature data, Kratosija appeared earlier and was introduced into cultivation quite earlier than Vranac. It is known that old varieties show heterogeneity in terms of expression of their properties which leads to gradual degradation of the variety and emergence of a number of biotypes within the population with noticeable differences. In contrast to Vranac, which has stable morphological characteristics and has undergone expansion in recent years and become the main grape variety for red wines, not only in Montenegro, but also in neighbouring countries (Herzegovina, Macedonia, Dalmatia), Kratosija is a heterogeneous variety with a number of biotypes.

The research was conducted in order to gather all available literature data on the origin and growing tradition of Vranac and Kratosija in Montenegro. With exploration of the total variability of populations of these varieties and values of certain biotypes and with selection of the best vines within them (vines that stand out with high yield, grapes quality, good habit, which are visually healthy and not infected by viruses), the work on clonal selection of these varieties has started. To increase the production of grapes and improve agrobiological, economic and technological characteristics of grapevine, the methods of individualised selection and clonal selection are being applied increasingly along with hybridisation.

Materials and methods

The work on selection of Montenegrin autochthonous varieties was done in two phases. The first phase of the research was implemented until 2004 on the experimental field of the Biotechnical Institute in Podgorica. It included the study of population variability of Kratosija variety, collection of its biotypes which differ and are grown all over Montenegro under various names and synonyms (Ulicevic 1966, Pejovic 1988). All collected biotypes under original name (Kratosija Velja, Kratosija Mala, Kratosija, Kratosija Srednja, Crni Krstac, Ljutica, Vrancina, Vran, Vranac, Vrancic, Kratosija with deep notches, Velji Vranac, Srednji Vranac, Kratosija or Vran, Bikaca, Cestozglavica, Rehuljaca) were planted on the experimental field in Ljeskopolje, on the rootstock K 5BB, grown in the same shape of trunk (double horizontal cordon) and the same conditions. In continuous research throughout the period 1996-2004, out of each biotype of Kratosija, 10 vines of every biotype were studied. Vines were pruned in the shape of double horizontal cordon, with the load of 24 buds per vine and 9.6 buds per m² of surface, respectively. Every vine represents one repetition. Quality of grapes and wine in Kratosija biotypes (number of bunches, weight of a bunch, length and width of a bunch and a berry, content of sugar and acids in the must as well as organoleptic score of wines produced from the studied biotypes of Kratosija) were monitored. Statistical analysis was performed so as to analyse variance for two-factorial experiment. Significance of differences was determined by LSD test on the level 0.05 and 0.01. The second phase of work on clonal selection included studies in the period 2004-2011. The research was conducted at Cemovsko field, on the vineyards of the "13. jul Plantaze" Company.

By visual observation of the variety population during the growing season, vines that stood out by their characteristics were selected. The sanitary status of the population was analysed by ELISA, PCR and indexing. Testing on viruses was conducted on 145 vines of Vranac variety and 31 vines of Kratosija variety. As regards Vranac population, 5 vines passed the complete sanitary control. Selected vines that passed sanitary control were propagated and planted in the nematode-free soil. During 2009 and 2010, all potential clones and variety population were observed and their agrobiological, economic and technological characteristics were compared. The quality of wines of potential clones was examined by applying standard methods and wine was produced in the microvinification cellar of the "13 jul Plantaze" Company.

Results and discussion

Research of Vranac and Kratosija

The old autochthonous Montenegrin grape varieties Vranac and Kratosija have been researched and described by many authors. M. Plamenac (1891) points out that Crmnica's wine is the best in Montenegro and can be compared with wines from Bordeaux. Jergovic (1892) in his publication in ‘’Montenegrin Voice’’ states that wine from Crmnica was made from Vranac and Kratosija, and it stood out by its quality. The first remarkable description of Kratosija variety was made by Petar Plamenac in the Ampelography Viale and Vermonela (1910). According to the documents of the Ministry of Internal Affairs of the Principality of Montenegro dating from 1905, description of Kratosija variety was made by M.Vujovic and P.Vojvodic. Stojanovic (1929) points out that Montenegro has vineyards. As for production of red wines in Montenegro, the author lists varieties Vranac and Kratosija. Also, he points out that the best vineyards in Montenegro are situated in Crmnica which is famous for the red wines that can reach high quality, while wines produced on Montenegrin coast are similar to wines from southern Dalmatia. Bulic (1949) gives broader ampelographic description of Kratosija (Grtosija, Grakosija and Kratkosija) from nine municipalities on Montenegrin coast, pointing out that "something" is present in Dalmatia, that is planting in Herzegovina and Montenegro and "that presumably came from these regions to Dalmatia".

Ulicevic (1959) states that characteristic variety of Crmnica viticultural area is Vranac, covering about 40% of the assortment of grape varieties. It is in proper sense a variety of Crmnica. The other variety which is almost as remarkable is Kratosija. Regarding the fact that it prevails in all vineyards older than 60-70 years, that its share is often 90% of the assortment of the grape varieties in other viticultural areas of this sub viticultural area and that it is grown in coastal and older plantations more, it may be concluded that it is the leading and probably the oldest Montenegrin variety. It is from both these varieties that the most appreciated and famous Crmnica’s red wine is made. They represent our autochthonous and original material for the production of high quality red wine since they have not been grown outside Montenegro, except perhaps, in the assortment and trial plantations. The

author claims that the oldest and most spread Californian variety Zinfandel is identical to our Kratosija. Its origin has not been determined, so it is most likely that it originates from these areas, where our emigrants could carry it. Ulicevic (1966) stated that the growing area was not wider than 30 km for Vranac and 100-150 km for Kratosija fifty-sixty years ago. Thus, Ulicevic states three types of Kratosija: Obicna Kratosija (Srednja Kratosija, Srednji Vranac, Ljutica), the type which has the greatest bearing potential and which is the most spread and of the greatest importance, Slabo Rodna Kratosija – the type you may find with the synonyms: Black Krstac, Vranj, Krstac, Vrancina which bears irregularly and the third type of Rehuljava Kratosija (Rehuljaca) - which has a morphologically regular flower but bears very loose clusters. Prof. D. Nastev (1967) states that Vranac variety (synonyms: Vranac Crnogorski, Vranac Prhljavac) is a Montenegrin variety, spread in Crmnica and on the Montenegrin coast. It was transferred to Macedonia and planted in 1950 in the trial field (Butel) first for the research under their conditions and, later, it expanded. Cetkovic (1975**)** states that Vranac and Kratosija are grown in the basin of Lake Skadar where very good quality wines are made. Zirojevic (1979) states that Vranac is an autochthonous variety that probably appeared by natural crossing from seeds. It is mostly represented in the basin of Lake Skadar, but lately, it has spread to Macedonia. According to Pejovic (1988), Kratosija in Crmnica, depending on the properties, is known as: Velja (big), Srednja (middle) and Mala (small), while in other viticultural areas of Montenegro, it is known as: Crna Vinogradarska, (Beri & Doljani), Crna Gorska, Srednji Vranac, Vrancina (Ljesanska & Rijecka ahija), Krstac, Vranja, Krstan, Ljutica (in Zagarac), etc. Based on the Project results in the period 1985/89/90, the author classified Kratosija into four basic variants, as follows: Velja Kratosija**,** which depending on the area from which it originates, has the synonym, namely, the assimilated title: Velji Vranac, Vran Krstan, Crni Krstac, Vrancina and Bikaca; - Srednja Kratosija, which has the title Middle Vranac, Vrancic, Ljutica, Obicna Kratošija, Cestzglavica; Mala Kratosija which has petty clusters and more balanced grape ripening; Rehuljaca, represented to a lesser degree and may be found as single grapevines within other variants and it has loose clusters. Avramov (1988) states that Vranac and Kratosija are autochthonous grapevine varieties of Montenegro. According to this author, Kratosija can be found under different names – synonyms such as: Gratosija, Grakosija, Kratkosija, Kratkosica and similar. A lot of clones were noticed within the variety. As for Kratosija, Cindric (1994, 2000) states that Vranac and Kratosija are autochthonous Montenegrin varieties and Kratosija is susceptible to grey rot and gathers less sugar and more acids in the grapes compared with Vranac variety. As for Buric (1995), Kratosija is an autochthonous grapevine variety of Montenegro, grown in Montenegro. Blended with Vranac, it is used for production of quality wines. These varieties complement each other perfectly- Kratosija always has more total acids in the grapes (sometimes sugar, as well) and Vranac has more colloidal substances. Bozinovik (1996, 1998) points out that Kratosija gives grapes for production of quality red wines. He recommends blending it with Vranac in the amount of 5%. He also states the existence of three variants of Kratosija: Kratosija Standard, Kratosija Rehuljava and Kratosija Neoplodjena. Vesna Maras (2000) has

classified 17 studied synonyms of the Kratosija variety into three variants-biotypes of Kratosija: Velja Kratosija, Srednja Kratosija and Rehuljaca. Milosavljevic (2008) states that Vranac is an auchthonous Montenegrin variety grown for centuries in Montenegro, adding it is likely that the crossing occurred spontaneously or as a result of spontaneous mutation. Except in Montenegro, it is grown in Herzegovina, Dalmatia, Macedonia and Metohia.

Genetic identification of varieties

As a result of cooperation between the "13. jul Plantaze" Company and Italian partners (Instituto sperimentale per la viticulture Susegana-Conrgliano TV), genetic identification of autochthonous Montenegrin vine varieties has been done. Originality has been confirmed for Vranac, Krstac and Zizak. Analyses have showed that Montenegrin autochthonous variety Kratosija has an identical genetic profile as Zinfandel from California, Primitivo from Italy and Crljenak Kastelanski from Croatia (Callo *et al.* 2008). In the same study, a DNA analysis showed a close relationship between Vranac and Kratosija and that there is the first level of relation (the closest relation).

Bearing in mind the importance of autochthonous varieties and exciting of germplasm for vine-growing and wine-producing sector of Montenegro, international projects are also implemented with the aim of identification of autochthonous varieties and other domesticated grapevine varieties, testing the variability of their population along with preservation and protection our genetic resources. All these activities are implemented through the following projects: SEEDNET project (2009-2010): "Identification, characterisation and conservation of old and autochthonous vine varieties in Eastern European countries"; SEERA NET 91/01 Project: "Preservation and establishment of true-to-type and virus free material of endangered grapevine cultivars in Croatia and Montenegro" and COST action: "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding".

Work on clonal selection of varieties

I phase - In order to investigate variability of Kratosija variety population and values of certain biotypes in the period of 1996/1998, 17 varieties-biotypes of Kratosija were collected on the experimental field of the Biotechnical Faculty (Maras 2000, 2004).Within these biotypes, the best vines (based on the number of bunches, weight of a bunch, yield of grapes, vine productivity and quality of grapes) were selected. They served as the endpoint mother plantation for further propagation and studies (Tab. 2). The selected vines were propagated and planted on the Experimental field of Biotechnical Institute in 2000-2004. In that period, classified vines were tested on viruses and showed high level of infection so they did not pass ELISA and PCR tests (18 vines including the selected vine from the population: 8 vines (44.44%) were infected with LR1+LR3; 6 vines (33.33%) with GVA+LR3; 2 vines (11.11%) with FL+GVA+LR3 and 2 vines (11.11%) with LR1.

In the period of 2004-2011, along with genetic identification, we started the second phase of the selection of our autochthtonous grape varieties.

By visual observation during the year, vines with emphasised yield, quality and good vegetative potential were selected from the population and subjected to sanitary control ELISA, PCR and indexing. Testing on viruses was conducted on 145 vines of Vranac variety and 13 vines of Kratosija variety. Most samples of Vranac variety were infected - 55 vines (37.93%) by LR3 and 45 vines (31.03%) with GVA+LR3. The vines were not infected with AR. The presence of virus with ELISA test was not identified in 17 vines (11.72%). The vines which passed sanitary control by ELISA test were tested with PCR. Out of 17 tested vines, 5 vines (29.41%) were infected with RSP and the same percentage (29.41%) with RSP+NN. Out of the total number of tested samples (17), 4 samples (23, 53%) passed PCR and indexing and virus was not identified. They represent very useful material which entered the process of individual clonal selection. Out of 13 tested vines of Kratosija variety, none passed sanitary control.

Tab.1. Quality of grape and wine of kratosija biotypes (average for 1996-1998)
Kvalitet grozdja i vina biotipova kratosije (prosjek za 1996-1998)

Biotip (A)	Broj grozdova	Masa grozda (g)	Dužina grozda (cm)	Širina grozda (cm)	Dužina bobice (mm)	Širina bobice (mm)	Prinos po čokotu (kg)	Udio šećera (%)	Udio kiselina (g/)l	Organoleptička ocjena vina
velja kratošija	16.30	332.23	18.66	11.72	14.54	15.05	5.44	21.67	7.37	17.50
velji vranac	34.77	188.00	18.21	11.40	14.52	14.86	6.48	22.13	6.83	17.36
crni krstač	16.90	290.30	18.14	11.65	14.68	14.58	4.84	22.03	6.77	17.39
vrančina	14.33	290.94	19.16	12.53	15.82	16.10	4.17	21.00	6.83	17.06
bikača	17.93	262.45	16.03	9.76	15.48	15.56	4.62	22.83	7.07	17.48
vran	12.90	302.53	18.39	11.65	15.36	15.16	3.85	23.47	7.03	17.70
srednja kratošija	19.07	274.08	17.51	11.72	15.39	14.96	5.30	21.87	6.93	17.13
kratošija ili vran	18.43	292.66	18.03	11.14	14.58	14.68	5.36	21.93	7.53	17.33
srednji vranac	26.67	229.09	18.29	11.60	13.84	13.73	5.97	20.40	7.30	17.27
vranac	12.47	418.73	18.88	12.54	14.72	14.55	4.61	21.33	7.20	17.29
vrančić	17.77	304.93	19.79	13.89	11.97	11.93	5.47	20.23	7.67	17.07
ljutica	17.00	260.73	19.02	12.55	12.60	12.54	4.27	20.03	7.80	16.93
kratošija	16.37	284.58	18.33	11.59	14.29	14.22	4.58	21.77	6.53	17.70
čestozglavica	20.83	297.72	18.89	11.97	14.02	14.05	5.37	21.63	6.63	17.26
kratošija mala	17.83	317.55	17.18	12.08	13.11	12.94	5.63	21.43	6.97	17.19
krat.sa dub. urez.	14.83	277.39	18.38	11.58	13.65	13.65	4.15	21.77	7.40	16.87
rehuljača	26.10	118.48	18.11	8.72	11.98	12.12	2.92	17.10	7.80	16.62
X	19.03	278.96	18.29	11.65	14.15	14.16	4.89	21.33	7.16	17.24
Godina I (B)	12.17	292.22	18.30	11.71	14.73	14.71	3.21	22.26	6.84	
Godina II (B)	18.49	272.96	18.35	11.74	14.06	14.13	4.69	21.66	7.05	
Godina III (B)	25.90	271.71	18.23	11.51	13.66	13.64	6.75	20.08	7.59	
(A) $LSD_{0.05\ 0.01}$	3.20 4.20	33.25 43.69	1.36 1.80	1.13 1.49	0.84 1.11	0.85 1.13	0.91 1.20	0.93 1.26	0.58 0.78	
(B) $LSD_{0.05\ 0.01}$	1.34 1.75	13.97 18.35	0.57 0.76	0.48 0.62	1.34 1.75	13.97 18.35	0.38 0.51	0.39 053	0.24 0.33	
(AxB) $LSD_{.05\ 0.01}$	5.54 7.28	57.58 75.67	2.36 3.12	1.96 2.58	5.54 7.28	57.58 75.67	1.58 2.08			

The separated vines of Vranac population that passed sanitary control (ELISA, PCR, indexing) were propagated and planted in 2008 and 2009 at the locality ''Nikolj Crkva'' (the soil passed control on the presence of nematodes). Propagated mother vines were planted and plantation of pre-base category which would serve for the studies of potential clones and their recognition of clones was formed. After the first crop during 2009 and 2010, all potential clones and the

variety population were observed and their agrobiological, economic and technological characteristics were compared. Processing of grape and wine making using potential clones were done in a microvinification cellar of the "13 jul Plantaze" Company. Produced wines were analysed and their sensory assessment was done. They surpassed the population of the variety by the quality of wine. In table 3, two-year results of grapes and wine quality for clones candidates are compared with the variety population.

Tab.2. Selected vines within studied biotypes (average for 1996-1998)
Odabrani cokoti unutar ispitivanih biotipova (prosjek za 1996-1998)

Biotype	Selected vine num.	Number of bunch	Weight of bunch (g)	Yield of grape (kg)	Weight of pruned vine (kg)	Productivity of vine (kg)
velja kratošija	10	21.00	456	9.60	1.13	10.73
velji vranac	6	38.33	238	8.94	1.96	10.90
crni krstač	9	22.00	313	6.74	0.88	7.62
vrančina	5	18.67	290	5.60	1.44	7.04
bikača	8	21.33	364	7.60	1.26	8.86
vran	8	18.33	299	5.65	1.40	7.05
srednja kratošija	8	25.00	310	7.74	0.98	8.72
kratošija ili vran	10	24.33	260	6.52	0.69	7.21
srednji vranac	3	34.33	220	7.53	1.25	8.78
vranac	9	16.33	408	6.09	1.20	7.29
vrančić	2	25.00	324	8.20	0.93	9.13
ljutica	7	20.33	266	5.14	0.48	5.62
kratošija	8	17.00	362	6.33	1.33	7.66
čestozglavica	1	32.33	259	7.62	1.18	8.80
kratošija mala	4	25.00	299	7.55	1.09	8.64
kratošija sa dub.urezima	3	21.00	263	5.99	1.40	7.39
rehuljača	4	32.67	115	3.67	1.33	5.00

Tab.3. Yield and quality of grape and wine of potential clones of vranac variety (2009-2010)
Prinos i kvalitet grozdja i vina potencijalnih klonova sorte vranac (2009-2010)

Parametri / Potencijalni klonovi	Broj grozdova po čokotu	Masa grozda (g)	Dužina grozda (cm)	Širina grozda (cm)	Dužina bobice (mm)	Širina bobice (mm)	ŠIRA			VINO				
							Šećer (%)	pH	Ukupne kiseline (g/l)	Alkohol (vol%)	Ukupni ekstrakt (g/l)	Ukupne kiseline (g/l)	Antocijani (mg/l)	Ukupni polifenoli (g/l)
	X	X	X	X	X	X	X	X	X	X	X	X	X	X
NC V 1	17.7	232.3	12.0	8.1	16.3	14.5	23.25	3.76	5.00	13.64	27.25	5.20	675	3.24
NC V 2	17.3	306.7	17.0	9.3	15.3	14.3	21.35	3.75	4.52	13.10	26.35	4.90	605	2.58
NC V 3	17.2	238.5	15.2	7.9	14.4	13.3	22.05	3.79	5.08	13.63	28.50	5.11	681	2.60
NC V 4	21.3	299.2	17.8	9.7	15.0	13.7	22.80	3.77	4.37	13.61	27.10	4.91	598	3.23
NC V 5	19.1	304.6	16.2	9.1	15.4	14.2	22.70	3.72	4.83	13.47	28.15	5.12	719	3.03
VRANAC POPULACIJA	21.7	229.5	16.9	9.4	16.5	15.2	21.65	3.55	4.99	13.60	28.55	6.33	591	2.92

The NCV 4 candidate had the highest number of bunches per vine and the NCV 2 candidate had the highest weight. The highest content of sugar was measured in a potential clone NCV 1. Two potential clones, namely NCV 1 and NCV 4, stood out in terms of the wine quality.

Conclusion

The testing results of 17 biotypes of Kratosija population showed variability in the variety as well as in its certain biotypes. Within the certain biotypes of vines, the best ones were separated to be used as a mother plantation for further propagation and studies according to the target selection.

Genetic identification has confirmed the authenticity of Montenegrin varieties Vranac, Krstac, Zizak, and it was confirmed for Kratosija to have an identical DNA profile as Zinfadel. Out of the selected and tested vines of Vranac population, only 5 vines have passed sanitary control and started the process of clonal selection. Two clone candidates, NCV 1 and NCV 4, surpassed the population of the variety in terms of the quality of grapes, must and wine.

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Istraživanje porijekla i rad na klonskoj selekciji crnogorskih sorti cv. vranac i cv. kratosija

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Sažetak

Vinogradarsko vinarska proizvodnja Crne Gore uglavnom se zasniva na gajenju autohtonih sorti vinove loze. Pored vranca, značajno mjesto u crnogorskom autohtonom sortimentu ima i sorta kratošija. Kratošija je autohtona crnogorska sorta nastala ranije i, prema mnogim literaturnim podacima, uvedena u kulturu gajenja znatno prije vranca. Danas je u vinogradima Crne Gore malo zastupljena zbog heterogenosti populacije, pa se uglavnom nalazi u kombinaciji sa sortom Vranac. U radu su prikazani rezultati istraživanja porijekla vranca i kratošije. Prikazana je genetička identifikacija koja je potvrdila autohtonost vranca dok je utvrđeno da autohtona sorta kratošija ima isti genetski profil kao cv. Zinfandel. U radu su prikazani višegodišnji rezultati rada na ispitivanju varijabilnosti njihovih populacija i rada na klonskoj selekciji. Izdvojeni su matični čokoti i podignuti predbazni i bazni zasad potencijalnih klonova sorte Vranac. Ostvareni rezultati u pogledu ispoljavanja agrobioloških i privredno tehnoloških pokazatelja ukazuju na potrebu daljeg rada na selekciji ovih sorti.

Ključne riječi: porijeklo, varijabilnost populacije, vranac, kratošija, klonska selekcija

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