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Ampelographic and Genetic Characterization of Montenegrin Grapevine Varieties

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Abstract

Montenegro is a small country in Balkan Peninsula with very long tradition of grapevine growing and wine making that originate from the pre-Roman period. Dominant place in Montenegrin viticulture belongs to autochthonous grapevine varieties Vranac, Kratosija, and Krstac, while in minor part, the other varieties are presented. Among many literature sources, the oldest historical document that pointed out the importance of autochthonous varieties is The Medieval Statute of Budva from fifteenth century. In order to better present Montenegrin germplasm, this research provides an overview of literature, ampelographic, and genetic analysis on autochthonous and domesticated varieties. Achieved results showed an important breeding history of grapevine and a large number of unique DNA profiles. Montenegro has the richness of grapevine diversity that can significantly enrich the diversity of vines in Europe.

Keywords: autochthonous varieties, Montenegro, grapevine diversity, genetic characterization, ampelographic characterization

1. Introduction

Montenegro is a small country placed in the Balkan Peninsula with one part overlooking the Adriatic Sea, right across the Italian region Puglia. A long tradition of grapevine growing in Montenegro is very well known and it dates back before the Roman period [1]. On the Montenegrin territory, a large number of tombstones with grapevine and wine motives which originated in ancient era were found. Found decorations were in the vine form and were directly related to the Dionysus god cult [2]. Numerous archeological sites and found objects that originated from the Illyrian period indicate that the wine was much appreciated and was quite used as the beverage. In the middle century, vine growing and winemaking were well developed in Montenegro and one of the oldest written documents that point out the importance of grapevine cultivation and importance of autochthonous varieties on the territory of today's Montenegro is the Medieval Budva's Statute from fifteenth century. After all, more organized work started during the reign of the King Nikola Petrović (1860–1918), who introduced the grape-growing and winemaking regulations. Within the grape varieties that have a long tradition of growing in Montenegro, the dominant place belongs to autochthonous grape varieties Vranac

and Kratošija that are used for making red wines, and for production of white wines, the Krstač was the dominant one [3, 4], while in a minor part, the other varieties were also presented. A major turning point in the development of Montenegrin viticulture was the realization of the project Čemovsko polje since 1977–1982, during which 1500 ha of vineyards were planted and modern wine cellar with 2 million liters capacity was established. However, as autochthonous grape varieties were in that time the most important, they also now constitute the viticulture and winemaking sector of Montenegro. In fact, Vranac grape variety represents more than 70% of total production and promotes Montenegro as an important wine country. In order to better present the germplasm of grapevine varieties in Montenegro, beside literary research, ampelographic and genetic analysis of autochthonous and domesticated grapevine varieties was done in order of their secure identification.

2. Materials and methods

Multi-year research included work on autochthonous and domesticated grapevine varieties in Montenegro. A detailed review of available literature and writing of earlier and contemporary authors regarding autochthonous and domesticated grapevine varieties was done. For a better understanding, we did also ampelographic and genetic analysis of selected varieties. Analysis included 188 samples of old representative vines aged between 50 and 300 years that are grown in affirmed vineyards in Montenegro. These studies also included 17 biotypes of the Kratošija variety (**Figure 2**) that were collected in 1987 in an experimental field in Ljeskopolje-Podgorica.

2.1 Ampelographic description

Ampelographic analysis, that is, a method of describing characteristics of grapevine varieties, was done with codes—a descriptor prescribed by O.I.V. (*Office International de la Vigne et du Vin*)—International Wine and Wine Office [5]. Observations were made on young shoots (OIV-003 and -004), young leaves (OIV-051 and -053), mature leaves (OIV-067, 068, 070, 076, 079, 080, 084, and 087), flowers (OIV-151), shoots (OIV-155), bunches (OIV-202, 204, 206, and 208), berries (OIV-220, 223, 225, 235, and 236), and, when possible, on must quality (OIV-505, 506, and 508). Ampelographic description also was done for 17 Kratošija biotypes (with following OIV codes: 003, 004, 016, 065, 068, 076, 079, 084, 085, 151, 202, 203, 204, 206, 220, 223, 225, 231, 235, 236, and 241).

2.2 Genetic analysis

For genetic analysis, DNA was extracted from young leaves. In the first phase of research, genotyping was performed with 11 SSR loci for variety identification: VVS2 [6]; VVMD5, VVMD7, VVMD27 and VVMD28 [7, 8]; VrZAG62 and VrZAG79 [9]; ISV2, ISV3 and ISV4 [10]; and VMCNG4b9 [11], as described by Ref. [12].

During the second phase of research, genotyping was done with nine microsatellite loci: VVS2 [6]; VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, and VVMD32 [7]; and ssrZAG62 and ssrZAG79 [9] as proposed by the GrapeGen06 consortium and by the European Vitis Database [13].

3. Results and discussion

3.1 Literature survey

The first mention of Montenegrin grapevine varieties was in fifteenth century [14], and later they were studied and described by many authors. Early mentioning of autochthonous Montenegrin grapevine variety was done by M. Plamenac [3]. He stated that in Montenegrin grape growing region Crmnica, Kratošija, Vranac, Krsmać, Sjerovina, Lisica, and Muskacelica varieties were grown. But the first more significant description of varieties Vranac and Kratošija was given by P. Plamenac [15]. All authors from the former Yugoslavia [16–29] reported Vranac and Kratošija as Montenegrin autochthonous grapevine varieties. Moreover, they stated that Vranac and Kratošija were grown only in Montenegro. From Montenegro, these varieties were spread to Macedonia [20] and Dalmatia [17] and to other countries in the former Yugoslavia. The Macedonian professor Nastev [20] states that Vranac is a Montenegrin autochthonous grapevine variety mostly cultivated in the Skadar lake region (Crmnica), but also in the Montenegrin seacoast. This author declares that Vranac has been transferred in the 1950s in Macedonia (experimental field Butel), from where it has been spread out through the former Yugoslavia. Montenegrin academic Ulicevic [18, 19] states that Vranac is a characteristic variety of vine growing area Crmnica in famous vine growing region Skadar lake, which occupies about 40% of the assortment. According to the same author, this is the only vine growing area where this variety is dominant and the growing area was not wider than 30 km.

The earliest reference of the Montenegrin variety Kratošija is reported in the Budva's Medieval Statute [14] in fifteenth century (1426–1431). In particular, it mentioned the "Kratošija's vineyards" indicating the importance of the Kratošija variety in that time in Budva (Montenegro). The Dalmatian ampelographer Bulić [17] described Kratošija (also considering the synonyms Gartošija, Grakošija, and Kratkošija) from nine municipalities of the Montenegrin coast (Budva, Grbalj, Luštica, Krtole, Kotor, Paštrovići, Prčanj, Tivat, and Herceg Novi). Moreover, the author stated that this variety was rarely found in the Dalmatia region where it was likely spread over from Montenegro. For the Kratošija variety, Ulicevic [18, 19] states that, it is strongly dominated in all plantations older than 60–70 years in that period and made 90% of the assortment in other regions. According to Ulicevic [18], the growing area of Kratošija was between 100 and 150 km through Montenegro and that is the main and probably the oldest Montenegrin variety.

In addition to very long Kratošija growing in Montenegro, there is also a huge heterogeneity of its population and it was described by many authors. M. Plamenac [3] for the first time mentioned biotypes of Kratošija and described some kind of Kratošija whose clusters are not compacted, but loose and it was called Reavica. Authors [16, 17, 19, 23, 24, 25, 30, 27, 28] also described different Kratošija's biotypes. Ulicevic [18] mentioned three types of Kratošija: Obična Kratošija, Slaborodna Kratošija, and Rehuljava Kratošija. Bozinovik et al. [30] stated that Kratošija has a high number of biotypes and described three of them (Kratošija standardna, Kratošija rehuljava, and Kratošija neoplodjena). The variability of the Kratošija population in Montenegro was also studied by Pejovic [24] and Maras [27]. Ampelographic analyses [27] were done on 17 biotypes of Kratošija, which are known under different names in viticultural areas in Montenegro: Velja Kratošija, Velji Vran, Crni Krstač, Vrančina, Bikača, Vran, Srednja Kratošija, Kratošija or Vran, Srednji Vranac, Velji Vranac, Vrančić, Ljutica, Kratošija, Čestozglavica, Kratošija mala, Kratošija sa dubokim urezima, and Rehuljača.

Krstač was dominant among the white grapevine varieties used for white wine production. Its name comes from the look and shape of the bunch that resembles a cross [15, 31]. Ulicevic [18] wrote that Krstač was believed to be autochthonous of Montenegro and probably originated from Beri (near Podgorica) with a growing area of 40–50 km. The same author stated synonyms for Krstač, in Doljani it was called Krstača bijela, in Vražegrmci Bijeli Krstač and in Beri Bijela vinogradarska.

Beside the most important grapevine varieties for viticulture in Montenegro Vranac, Kratošija, and Krstač there are also some literature data about minor grapevine varieties.

Žižak or Žižak bijeli [17] is considered another autochthonous variety of Montenegro and its origin is unknown. Individual vines can be found nearby Podgorica, but it is mostly grown on the Montenegrin seacoast (Boko-Kotorski sub region). Ulićević [18] also described Žižak as an important variety from which, in some places in Boka, are produced dessert wines called Prošek.

Ulicevic [18] states that Čubrica is used for red wine production and is represented in very small percentage in the vineyards of the Podgorica sub region (Doljani and Kuči). According to the author, vineyards in Doljani are quite old and

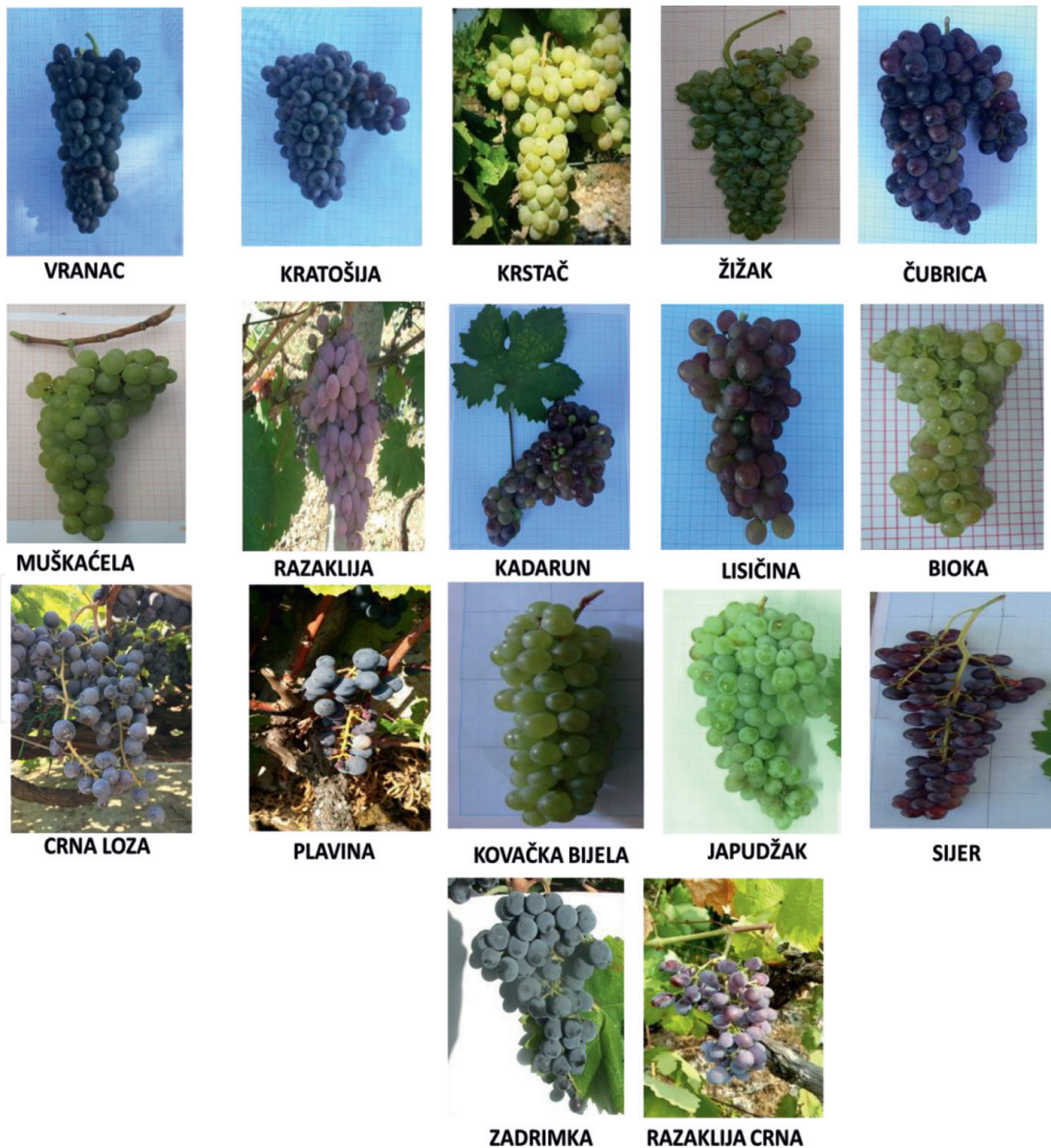


Figure 1.
Bunches of researched grapevine varieties.

none of the contemporary people, neither then nor now, did not know when these vineyards were planted.

Literature data about Muškačelica were given by M. Plamenac [3], and he stated that it is the little grown white grapevine variety that has very strong smell while the variety Sjerovina is russet grapevine variety that has round berries.

The first mention of Lisica was in Grlica [3], where the author reported that this variety is grown in Crmnica. About Lisičina, Stojanović [16] reports the use of this cultivar for white wine production in Montenegro. Bulić [17] and Ulićević [18] wrote that Lisičina (synonyms are Lisica, Ružica, Sjemerava, and Sjeruša) was grown in Montenegro around Bar, Ulcinj, Podgorica, and Virpazar (Crmnica).

Zadrimka was a major variety grown in the Ulcinj viticultural region in the late 1800s until phylloxera, and later World War II devastated the vineyards and almost drove the cultivar to extinction [19].

According to Ulićević [18], large areas under vineyards were in Bokokotorski subregion. Kadarun was dominated, while Kratošija and Vranac were less represented.

Ulićević [18] states that the Razaklija cultivar from both the Skadar Lake and the seacoast region constituted 95% of total table grape production in Montenegro. Many authors from Yugoslavia consider that Razaklija originates from Asia Minor [19]. It is not known how and when it arrived in Montenegro, Macedonia, and other countries and how it was spread.

3.2 Ampelographic descriptions

Ampelographic descriptors for certain varieties (**Figure 1**) already existed and through these researches data with some descriptions of additional varieties were fulfilled. Ampelographic descriptions of researched varieties, except Trojka, are given in **Table 1**.

Ampelographic descriptions are available for all Kratošija accessions listed in **Table 2** and grown at the Experimental Estate in Podgorica-Lješkopolje [27]. The name of each Kratošija biotype is in connection with some of its particular characteristics. Cluster weight was highly variable among accessions and correlated with morphology and these traits showed to be stable within each biotype [27].

3.3 Genetic identification

The varietal identification was achieved by comparing the obtained SSR profiles with available molecular databases and literature data. The work on the genetic identification of Montenegrin autochthonous varieties began in collaboration with *Istituto sperimentale per la viticoltura-Susegana-Conegliano-TV*. The analysis confirmed that Vranac, Krstač, and Žižak have an original DNA profile. Kratošija, an old Montenegrin grape variety, has an identical genetic profile as the Zinfandel from California, Primitivo from Italy, and Crljenak kaštelanski from Croatia [32]. The same authors also suggest a probable first degree relationship between Vranac and Kratošija. Research on Montenegrin grapevine diversity continued and further analysis of 70 samples revealed 14 different genotypes. The results showed already identified genotypes: Vranac, Kratošija, Krstač, and Žižak and 10 new identified genotypes [33]. In **Table 3**, SSR markers of identified varieties are presented. Also, all 17 biotypes of Kratošija were confirmed to have the same genetic profile as Zinfandel/Primitivo [33]. According to Maras et al. [33] the variety Muškačela is identical to a variety Muscat bianco–Muscat a petits grains. Trojka accession has the same profile as Muscat rouge de Madere (alias Moscato violetto), another important member of the Muscat family. Based on analysis, it can be concluded that Plavina is

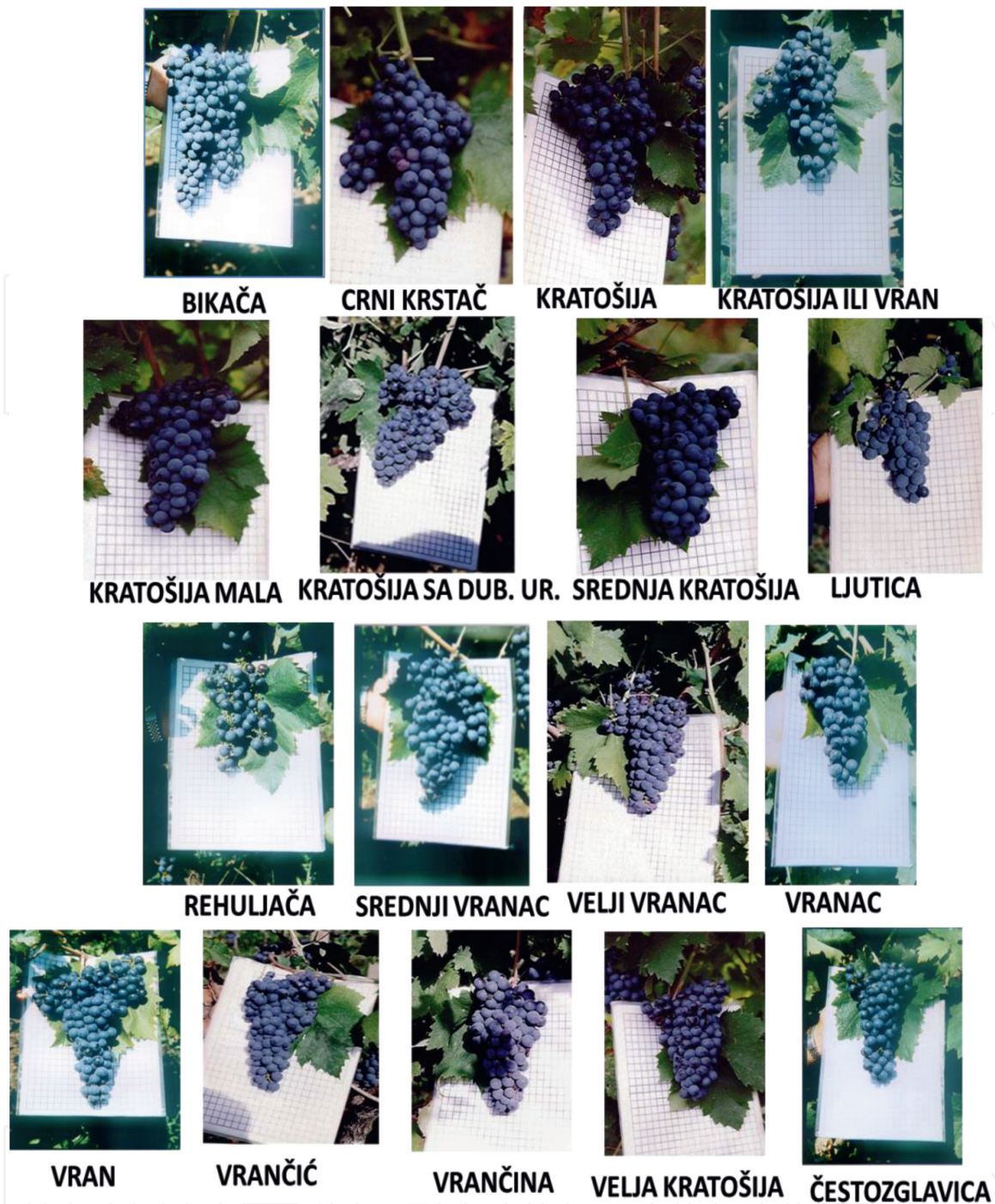


Figure 2. Bunches of Kratošija variety biotypes.

a progeny of Kratošija. Bioka shares the same genotype as the Italian Francavidda and Croatian Zlatarica Vrgorska. Comparing data with the European Vitis database in the Vitis International Variety Catalog [34] Kadarun is Reported as a Turkish cultivar. The red berry Razaklija accession that was analyzed matches the SSR profile of Crven Drenok [35]. Crna Loza, Čubrica, Lisičina, and Razaklija crna show unique SSR profiles. Crna Loza was considered as a Kratošija synonym, but analysis shows a different SSR profile for this variety. Based on SSR allele sharing at all analyzed loci, Razaklija crna could really be a progeny of Drenak Crven [33]. Bearing in mind the importance of grapevine germplasm in Montenegro, the research was continued through two international projects SEEDNet and SEE.ERA NET. As result of SEEDNET project, from 16 considered samples, 6 different genotypes were identified [36]. The identified varieties are Vranac, Kratošija, Krstač, Čubrica, Lisičina, and Razaklija. The variety Razaklija has the identical SSR profile

	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	
																					Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip
																					Young shoot: density of prostrate hairs on the shoot tip
																					Shoot: attitude (before tying)
																					Mature leaf: size of blade
																					Mature leaf: number of lobes
																					Mature leaf: shape of teeth
																					Mature leaf: degree of opening/ overlapping of petiole sinus
																					Mature leaf: density of prostrate hairs on main veins on the lower side of blade
																					Mature leaf: density of erect hairs on main veins on the lower side of blade
																					Flower: sexual organs
																					Bunch: length (peduncle excluded)
																					Bunch: width
																					Bunch: density
																					Bunch: length of peduncle of primary bunch
																					Berry: length
																					Berry: shape
																					Berry: color of skin
																					Berry: intensity of flash anthocyanin coloration
																					Berry: firmness of flesh
																					Berry: particular flavor
																					Berry: formation of seeds
Bilkača	3	4	6	65	68	76	79	86	87	151	202	203	204	206	220	223	225	231	235	236	241
Crni krstač	3	7	1	7	3-4	2	6	5	7	3	7	5	7	1	5	3	6	3	3	1	3
Kratošija	3	7	1	7	3	2	7	5	7	3	7	5	7	1	5	3	6	3	3	1	3
Kratošija ili Vran	3	7	1	7	3	2	7	5	7	3	7	5	7	1	5	3	6	3	3	1	3
Kratošija mala	3	7	1	7	3-4	2	7	5	7	3	5	5	9	1	5	3	6	3	3	1	3
Kratošija sa dubokim urezom	3	7	1	7	3-4	2	7	5	9	3	7	5	7	1	5	3	6	3	3	1	3
Kratošija srednja	3	7	1	7	3	2	7	5	7	3	5	5	7	1	5	3	6	3	3	1	3
Ljutica	3	7	1	7	3-4	2	6	5	7	3	7	5	7	1	5	3	6	3	3	1	3
Rehuljača	3	7	1	5	3	2	7	5	7	3	7	3	1	1	5	3	6	3	3	1	3
Srednji Vranac	3	7	1	7	3	2	7	5	7	3	7	5	7	1	5	3	6	3	3	1	3

	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	OIV	
																						Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip
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																						Berry: firmness of flesh
																						Berry: particular flavor
																						Berry: formation of seeds
	3	4	6	65	68	76	79	86	87	151	202	203	204	206	220	223	225	231	235	236	241	
Velji Vranac	3	7	1	7	3	2	7	5	7	3	7	5	7	1	5	3	6	3	3	1	3	
Vranac	3	7	1	7	3	2	7	5	7	3	7	5	7	1	5	3	6	3	3	1	3	
Vran	3	7	1	7	3	2	7	5	7	3	7	5	9	1	5	3	6	3	3	1	3	
Vrančić	3	7	1	7	3	2	7	5	9	3	7	5	7	1	5	3	6	3	3	1	3	
Vrančina	3	7	1	7	3	2	6	5	7	3	7	5	7	1	5	3	6	3	3	1	3	
Velja Kratošija	3	7	1	7	3-4	2	7	5	7	3	7	5	9	1	5	3	6	3	3	1	3	
Čestozglavica	3	7	1	7	3	2	7	5	7	3	5	5	7	1	5	3	6	3	3	1	3	

Table 2.
Ampelographic descriptions of Kratošija accessions [27].

Variety	VVS2	VVMD5	VVMD7	VVMD27	VrZAG62	VrZAG79	VVMD28	ISV2 (VNC671)	ISV3 (VMC6F1)	ISV4 (VMC6G1)	VMC NG4B9											
Vranac	133	133	226	226	247	249	181	181	193	199	258	258	239	251	151	165	133	139	177	177	164	172
Kratošija	133	143	226	236	247	249	179	181	199	203	236	258	251	261	141	165	139	139	177	177	150	164
Krstač	133	139	232	240	239	239	185	185	187	195	250	258	247	261	145	151	133	139	169	177	158	166
Žižak	143	145	240	240	239	263	179	191	187	193	250	250	251	261	141	159	136	139	183	187	150	152
Trojka	133	133	226	228	247	249	179	183	185	203	244	254	249	261	141	161	133	133	169	177	158	158
Čubrica	133	143	236	246	239	249	179	181	187	199	236	258	239	261	141	141	133	139	177	193	164	172
Muškaćela	133	133	228	236	233	249	179	294	185	195	250	254	249	271	141	143	133	139	163	187	158	166
Razaklija	139	143	232	246	239	247	181	185	185	187	250	258	239	261	141	143	133	145	177	193	150	176
Kadarun	143	145	232	236	249	249	179	181	193	199	258	258	249	261	165	165	139	139	177	193	150	150
Lisičina	133	137	238	246	239	239	181	185	187	195	250	250	237	239	141	143	133	139	177	197	158	172
Bioka	135	143	226	232	239	249	179	181	187	193	250	258	249	251	137	165	133	139	169	187	166	176
Crna loza	139	143	226	232	247	249	179	181	185	199	236	258	261	261	141	141	133	139	177	177	150	164
Plavina	133	143	232	236	239	249	179	189	187	199	236	242	251	261	143	165	139	139	177	177	150	152
Razaklija crna	135	139	232	238	247	255	181	185	185	203	250	258	239	247	141	165	139	145	177	187	158	176

Table 3.
SSR profiles of researched grapevine varieties in Montenegro.

to Drenok crveni from Macedonia. Within SEE.ERaNet project on various viticulture areas, from different vine growing regions, 96 samples of vines were selected and marked for identification. From these samples, 15 different genotypes were revealed some already known (Vranac, Kratošija, Krstač, Žižak, Čubrica, and Lisičina) and some with original SSR profile (Kovačka bijela, Sijer, and Zadrimka). Out of the six remaining samples, three accessions were found to be misnomers, one coincides with a previously identified variety in another country, and two accessions showed the original SSR profile which did not match any of the known varieties [37]. Accession of Japudžak from Montenegro is identical to the Turkish variety Yapıncak [38]. As a result of this project, in 2012, Montenegro presented and included its autochthonous and domesticated grapevine varieties in the EU Vitis database (Vranac, Kratošija, Čubrica, Krstač, Žižak, Japudžak, Sijer, Lisičina, Zadrimka, and Kovačka bijela). To preserve grapevine germplasm, the National collection of identified varieties was planted in Ćemovsko polje.

4. Conclusions

According to available literature and obtained results of ampelographic and genetic identification, Montenegro has a very long tradition of grapevine growing and very rich grapevine germplasm. There are varieties whose identification was done, but there are a lot of varieties with unknown origin and identity. Ampelographic description of 18 identified varieties as well as of 17 Kratošija biotypes was done and presented. During multiple years of research, genetic identification of 188 samples was carried out and the results revealed the original DNA profile for Vranac, Krstač, Žižak, Crna Loza, Čubrica, Lisičina, Razaklija crna, Kovačka bijela, Zadrimka, and Sijer. Kratošija, and every of its 17 biotypes, have the same DNA profile as Italian Primitivo, Californian Zinfandel and Croatian Crljenak Kaštelanski. Mušakaćela is Moscato bianco while Trojka is Moscato violeto. Montenegrin Bioka is the same as Italian Francavidda and Croatian Zlatica Vrgorska. The variety Razaklija is the same as Drenak crveni. For Japudžak the same SSR profile as for Turkish Yapıncak is discovered.

Research and work on autochthonous and domesticated grapevine varieties in Montenegro are of great importance for the viticulture and winemaking sector. Having in mind this and very interesting results achieved, it was necessary to continue with investigation of Montenegrin grapevine germplasm. Further research with partners from the Institute for Vine and Wine in La Rioja will be done with the aim of analyzing a large number of samples across Montenegro, and then determining its origin and genetic relationships (pedigree analysis).

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Conflict of interest

I declare I have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or nonfinancial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.


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