SSR MOLECULAR MARKER ANALYSIS OF THE GRAPEVINE GERMPLASM OF MONTENEGRO

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Abstract

Aim : Given that the information about the origin, genetic relationships, and diversity of Montenegrin grapevines is still partial, we performed a detailed analysis of the germplasm in this country using simple sequence repeat (SSR) markers. Our main goal was to determine the identity of cultivars unique to Montenegro and those shared with other countries, especially the neighbouring ones.

Methods and results : Seventy samples were collected and 14 genotypes were found. After SSR profile comparison with available molecular databases and literature data, the identity of each genotype was established. Five well-known cultivars were found, the others being minor, lesser-known cultivars.

Conclusion : This research provides an overview of the Montenegrin grapevine assortment. There are cultivars shared with other countries, mainly the neighbouring ones, while others are likely native to Montenegro. The Kratošija population (*alias* Primitivo, Zinfandel and Crljenak Kaštelanski) has a large number of different names in Montenegro and also a wide morphological variability. Therefore, Montenegro is the best candidate as the origin and spreading point of this cultivar.

Significance and impact of the study : The present study adds information on the identity, origin, diffusion and variability of some grapevine cultivars, allowing us to reconstruct the history and evolution of national and transnational ampelographic assortment of Montenegro.

Key words : SSR genotyping, Kratošija, Bioka, intra-varietal biodiversity, minor cultivars

Résumé

Objectif : Les informations disponibles sur l'origine, les relations génétiques et la diversité des variétés de vigne du Monténégro étant encore partielles, nous avons réalisé un inventaire du germoplasme de ce pays à l'aide de marqueurs SSR (répétitions de séquences simples). Notre objectif principal était de déterminer les variétés uniques dans le Monténégro et les variétés communes avec les autres pays, et en particulier avec ceux limitrophes.

Méthodes et résultats : Soixante-dix échantillons ont été prélevés et 14 génotypes ont été déterminés. L'identité de chaque génotype a été définie suite à la confrontation des profils SSR avec les bases de données moléculaires disponibles et les données fournies dans la littérature. Cinq cépages bien connus ont été identifiés, les autres sont des variétés mineures moins connues.

Conclusion : Cette recherche fournit un éventail de l'assortiment variétal viticole du Monténégro. Certaines variétés sont identiques à celles d'autres pays, en particulier ceux limitrophes, alors que d'autres sont probablement indigènes du Monténégro. La population de Kratošija (*alias* Primitivo, Zinfandel et Crljenak Kaštelanski) a un grand nombre de noms différents dans le Monténégro ainsi qu'une vaste variabilité morphologique. C'est pour cette raison que le Monténégro représente le meilleur candidat comme zone d'origine et de diffusion de ce cépage.

Importance et impact de l'étude : Cette recherche apporte de nouvelles informations sur l'identité, l'origine, la diffusion et la variabilité de certaines variétés de vigne, permettant ainsi de reconstruire l'histoire et l'évolution de l'assortiment variétal national et transnational du Monténégro.

Mots clés : génotypage SSR, Kratošija, Bioka, biodiversité intra-variétale, variétés mineures

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INTRODUCTION

Montenegro is a small country located in the Balkan Peninsula and overlooking the Adriatic Sea. Grapevine growing in Montenegro dates back before the Roman period (Vuksanović, 1977). The climatic, soil and orographic conditions of southern Montenegro define two wine regions: the Montenegro Skadar Lake basin, encompassing the Podgorica, Crmnica, Rijecki, Bjelopavlicki and Katunski sub regions, and the Montenegrin seacoast region, including the Boko-Kotorski, Budvansko-Barski, Ulcinjski and Grahovsko-Nudolski sub regions. Ancient and probably autochthonous cultivars dominate the present grapevine assortment of these two regions, and very heterogeneous populations appear as a consequence of perennial growing. Furthermore, there are numerous local grapevine cultivars lacking heterogeneity in their population, which have been registered as autochthonous even though they were not definitively identified. Most of these cultivars can be recognized using OIV codes, but it is not enough for their reliable identification (Ulićević, 1966; Pejović, 1988; Maraš, 2000).

Current viticulture and wine production in Montenegro is based mainly on presumed autochthonous grapevine cultivars, such as Vranac, Kratošija, Krstač, and Žižak. The dominant cultivars for red wine production are Vranac and Kratošija. Significantly less prevalent is Krstač, used for white wine production, while no commercial wine is produced from Žižak. The presence of Muškaćela and Trojka is significantly lower.

The results of Calò *et al.* (2008) showed that Kratošija is a synonym of the Californian Zinfandel, the Italian Primitivo and the Croatian Crljenak Kaštelanski, and suggested a first-degree relationship with the Montenegrin Vranac. Contrary to Vranac, which has stable morphological traits and has undergone expansion in the recent years, Kratošija is very ancient and a lot of biotypes have been found within its population, not fully investigated yet. Because of its heterogeneity, Kratošija is less present alone in the vineyards, and is found mostly in combination with Vranac, which dominates in the assortment of red grape cultivars. The heterogeneity of Kratošija has been described by many authors (Stojanović, 1929; Bulić, 1949; Jelaska, 1954; Ulićević, 1966; Avramov, 1988; Pejović, 1988; Burić, 1995; Bozinovik *et al.*, 1998; Maraš, 2000; Maraš *et al.*, 2004). It is well known that the features of an old grapevine cultivar may change with the accumulation and fixation of mutations in the shoot apical meristem, giving rise to many biotypes within the population, even with noticeable morphological or phenological differences. In this context, our work had different purposes. Using simple sequence repeat (SSR) markers, we wanted i) to investigate the identity of almost all grapevine cultivars grown in Montenegro; ii) to determine which cultivars are likely native to Montenegro and which are common to neighbouring countries; iii) to ascertain the identity of presumed Kratošija biotypes collected in the experimental field of the Biotechnical Institute in Ljeskopolje and coming from different Montenegro private vineyards, where they are cultivated under many different names, depending on the area; and iv) to verify the correct identity of Vranac mother vines chosen for clonal selection process.

MATERIALS AND METHODS

1. Plant material

Seventy samples were selected for genotyping during the period from 2006 to 2010. The vines were collected from different viticultural areas of Montenegro (Table 1).

2. Microsatellite DNA analysis

DNA was extracted from young leaves obtained from cuttings, and genotyping was performed with 11 SSR loci routinely employed at CRA-VIT (Centro di Ricerca per la Viticoltura, Italy) for cultivar identification: VVS2 (Thomas and Scott, 1993); VVMD5, VVMD7, VVMD27 and VVMD28 (Bowers *et al.*, 1996 and 1999); VrZAG62 and VrZAG79 (Sefc *et al.*, 1999); ISV2, ISV3 and ISV4 (Crespan, 2003); and VMCNG4b9 (Welter *et al.*, 2007), as described by Crespan *et al.* (2006). The varietal identification was achieved by comparing the obtained SSR profiles with available molecular databases and literature data.

3. Statistics on SSR data

To evaluate the discrimination power of the 11 SSRs used in this work, the following parameters were calculated: the polymorphic information content (PIC) and the combined non-exclusion probability for identity of unrelated and full-sib genotypes. SSR data were computed using 1418 unique profiles of *Vitis vinifera* L. cultivars present in the CRA-VIT molecular database, including the new genotypes found in the present work. CERVUS 3.0.3 software was used for calculations (Marshall *et al.*, 1998; Kalinowski *et al.*, 2007; http://www.fieldgenetics.com). Genotypes showing a single allele were considered as homozygous.

RESULTS AND DISCUSSION

Fourteen genotypes have been found within the 70 samples (Table 2). Using our set of 11 SSR markers, the mean PIC computed on 1418 unique

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Table 1 - Accession list (name, provenance and identity by genotyping)

ID	Accession name	Provenance (viticultural areas, municipalities)	Identity
1	BIKAČA	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošjia
2	BIKAČA	Kuci, Podgorica	Kratošjia
3	BIKAČA	Doljani, Podgorica	Kratošjia
4	BIOKA	Brceli, Crmnica, Bar	Bioka
5	CRNA LOZA	Doljani, Podgorica	Crna Loza
6	CRNI KRSTAČ	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošjia
7	ĆUBRICA	Kuci, Podgorica	Ćubrica
8	KADARUN	Herceg Novi	Kadarun
9	KRAT. SA DUB. UREZ.	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
10	KRATOSIJA	Brceli, Crmnica, Bar	Kratošija
11	KRATOŠIJA	Cemovsko polje, Podgorica	Kratošija
12	KRATOŠIJA	Cemovsko polje, Podgorica	Kratošija
13	KRATOŠIJA	Cemovsko polje, Podgorica	Kratošija
14	KRATOŠIJA	Crmnica, Bar	Kratošija
15	KRATOŠIJA	Doljani, Podgorica	Kratošija
16	KRATOŠIJA	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
17	KRATOŠIJA	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
18	KRATOSIJA	Nikolj Crkva, Cemovsko polje, Podgorica	Kratošija
19	KRATOŠIJA SREDNJA	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
20	KRATOŠIJA ILI VRAN	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
21	KRATOŠIJA MALA	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
22	KRATOŠIJA MALA	Ovtocic, Crmnica, Bar	Kratošija
23	KRSTAČ B.	Cemovsko polje, Podgorica	Krstač
24	KRSTAČ B.	Cemovsko polje, Podgorica	Krstač
25	KRSTAČ B.	Cemovsko polje, Podgorica	Krstač
26	KRSTAČ BIJELI	Cemovsko polje, Podgorica	Krstač
27	LISIČINA	Brceli, Crmnica, Bar	Lisičina
28	LISIČINA	Godinje, Crmnica, Bar	Lisičina
29	LJUTICA	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošjia
30	MUŠKAĆELA	Cemovsko polje, Podgorica	Muscat blanc à petits grains
31	MUŠKAĆELA	Cemovsko polje, Podgorica	Muscat blanc à petits grains
32	MUŠKAĆELA	Cemovsko polje, Podgorica	Muscat blanc à petits grains
33	MUŠKAĆELA	Nikolj Crkva Cemovsko polje, Podgorica	Muscat blanc à petits grains
34	PLAVINA	Herceg Novi	Plavina
35	RAZAKLIJA	Doljani, Podgorica	Drenak crven
36	RAZAKLIJA CRNA	Kuci, Podgorica	Razaklija crna
37	REHULJACA	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
38	SREDNJI VRANAC	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
39	TROJKA	Ljeskopolje, Biotechnical Institute, Podgorica	Moscato violetto/Muscat rouge de Madere
40	TROJKA	Ljeskopolje, Biotechnical Institute, Podgorica	Moscato violetto/Muscat rouge de Madere
41	TROJKA	Ljeskopolje, Biotechnical Institute, Podgorica	Moscato violetto/Muscat rouge de Madere
42	VELJI VRANAC	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
43	VRAN	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
44	VRANAC 9	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
45	VRANAC	Ovtocic, Crmnica, Bar	Vranac
46	VRANAC	Ovtocic, Crmnica, Bar	Vranac
47	VRANAC	Ovtocic, Crmnica, Bar	Vranac
48	VRANAC	Limljani, Crmnica, Bar	Vranac
49	VRANAC	Brceli, Crmnica, Bar	Vranac
50	VRANAC	Cemovsko polje, Podgorica	Vranac
51	VRANAC	Cemovsko polje, Podgorica	Vranac
52	VRANAC	Cemovsko polje, Podgorica	Vranac
53	VRANAC	Nikolj Crkva Cemovsko polje, Podgorica	Vranac
54	VRANAC	Nikolj Crkva Cemovsko polje, Podgorica	Vranac
55	VRANAC	Nikolj Crkva Cemovsko polje, Podgorica	Vranac
56	VRANAC	Nikolj Crkva Cemovsko polje, Podgorica	Vranac
57	VRANAC	Brceli, Crmnica, Bar	Vranac
58	VRANAC (clone 2/8)	Crmnica, Bar	Vranac
59	VRANAC (clone 6/8)	Crmnica, Bar	Vranac
60	VRANAC NCV 14/39	Cemovsko polje, Podgorica	Vranac
61	VRANAC NCV 15/13	Cemovsko polje, Podgorica	Vranac
62	VRANAC NCV 62/7	Cemovsko polje, Podgorica	Vranac
63	VRANAC NCV 62/13	Cemovsko polje, Podgorica	Vranac
64	VRANAC NCV 62/8	Cemovsko polje, Podgorica	Vranac
65	VRANCIC	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
66	VRANCINA	Ljeskopolje, Biotechnical Institute, Podgorica	Kratošija
67	ŽIŽAK	Cemovsko polje, Podgorica	Žižak
68	ŽIŽAK	Cemovsko polje, Podgorica	Žižak
69	ŽIŽAK	Cemovsko polje, Podgorica	Žižak
70	ŽIŽAK	Nikolj Crkva, Cemovsko polje, Podgorica	Žižak

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Table 2 - SSR profiles of grapevine varieties grown in Montenegro (allele lengths are in bp)

VARIETY	berry colour *	use **	VVS2		VVMD5		VVMD7		VVMD27		VrZAG62		VrZAG79		VVMD28		ISV2 (VMC6E1)		ISV3 (VMC6F1)		ISV4 (VMC6G1)		VMC NG4B9	
BIOKA	B	W	135	143	226	232	239	249	179	181	187	193	250	258	249	251	137	165	133	139	169	187	166	176
CRNA LOZA	N	W	139	143	226	232	247	249	179	181	185	199	236	258	261	261	141	141	133	139	177	177	150	164
ČUBRICA	N	W	133	143	236	246	239	249	179	181	187	199	236	258	239	261	141	141	133	139	177	193	164	172
RAZAKLIJA (Drenak crven)	R	T	139	143	232	246	239	247	181	185	185	187	250	258	239	261	141	143	133	145	177	193	150	176
KADARUN	N	W	143	145	232	236	249	249	179	181	193	199	258	258	249	261	165	165	139	139	177	193	150	150
KRATOŠIJA	N	W	133	143	226	236	247	249	179	181	199	203	236	258	251	261	141	165	139	139	177	177	150	164
KRSTAČ	B	W	133	139	232	240	239	239	185	185	187	195	250	258	247	261	145	151	133	139	169	177	158	166
LISIČINA	R	W	133	137	238	246	239	239	181	185	187	195	250	250	237	239	141	143	133	139	177	197	158	172
TROJKA (mutant of Moscato violetto)	B	W	133	133	226	228	247	249	179	183	185	203	244	254	249	261	141	161	133	133	169	177	158	158
MUŠKAĆELA (Muscat blanc á petits grains)	B	W	133	133	228	236	233	249	179	194	185	195	250	254	249	271	141	143	133	139	169	187	158	166
PLAVINA	N	W	133	143	232	236	239	249	179	189	187	199	236	242	251	261	143	165	139	139	177	177	150	152
RAZAKLIJA CRNA	N	T	135	139	232	238	247	255	181	185	185	203	250	258	239	247	141	165	139	145	177	187	158	176
VRANAC	N	W	133	133	226	226	247	249	181	181	193	199	258	258	239	251	151	165	133	139	177	177	164	172
ŽIŽAK	B	W	143	145	240	240	239	263	179	191	187	193	250	250	251	261	141	159	139	139	183	187	150	152

*B : white, N : black, R : red.

**W : wine, T : table.

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profiles of *Vitis vinifera* L. cultivars was 0.8036; the estimated probability of identity to deal with unique genotypes was 3.38E-15 for unrelated genotypes and 9.52E-6 for full sibs. These parameters showed the significant reliability of this set of markers for varietal identification.

The identification work showed that five genotypes match well-known cultivars, namely Kratošija, Muscat blanc à petits grains, Muscat rouge de Madere (*alias* Moscato violetto), Plavina and Vranac. In particular, the accessions named Kratošija (plus an adjective), Bikača, Ljutica, Rehuljaca, Srednji Vranac, Velji Vranac, Vran, Vrancic, Vrancina and a wrongly named Vranac (id 44 in Table 1) all belong to the Kratošija cultivar, as previously supposed based on ampelographic descriptions (Maraš, 2000). Crni Krstač matches the Kratošija profile and is another clear misnomer. The first remarkable descriptions of Vranac and Kratošija cultivars have been given by Petar Plamenac in the Ampelography of Viala and Vermorel (1910). Later on, many scientists (Stojanović, 1929; Bulić, 1949; Ulićević, 1959 and 1966) state that Vranac is present only in Montenegro. Ulićević (1959) writes the following: "characteristic cultivar of Crmnica from the Skadar Lake viticultural area is Vranac, covering about 40 % of the assortment of grape cultivars. As a matter of fact, this is the only viticultural area where it prevails and the only one where it is represented to a greater extent. It is in a proper sense a Crmnica cultivar". Nastev (1967) states that Vranac is a Montenegrin autochthonous grapevine cultivar mostly cultivated in the Skadar lake region (Crmnica), but also on the Montenegrin seacoast. This author declares that Vranac has been spread in the 1950s in Macedonia (experimental field of Butel). Nowadays, it represents the leading cultivar for red wine production in this country (Stajner *et al.*, 2009). Ulićević (1959) states that Kratošija is almost as remarkable as Vranac. In the same paper he writes that Kratošija dominated in Montenegrin vineyards for over 70 years and that it often represented 90 % of the assortment. Therefore, Kratošija is the main and probably the oldest Montenegrin grapevine cultivar. Ulićević (1959) argues that the Californian Zinfandel is identical to Kratošija and that Zinfandel likely originates from Montenegro and has been exported by Montenegrin emigrants. This was supported by the work of Calò *et al.* (2008), confirming that Kratošija has the same genetic profile as Zinfandel. Avramov (1988), Pejović (1988), Cindrić (1994), Cindrić *et al.* (2000), Burić (1995), Božinovik (1996), Maraš (2000) and Maraš *et al.* (2004) write that Vranac and Kratošija are autochthonous cultivars from Montenegro and that Kratošija has a very heterogeneous population. The wines of these cultivars are complementary: Kratošija usually provides a higher amount of total acids and sugars, while Vranac is richer in coloured substances. Bulić (1949) gives a description of Kratošija with synonyms (Gartosija, Grakosija, Kratkosija) from the nine municipalities of the Montenegrin seacoast. This cultivar is rarely found in the Dalmatia region, therefore, the author states that it has been possibly introduced to Dalmatia from Montenegro. By 2001, only about twenty living vines of Crljenak Kaštelanski had been found in Dalmatia (Maletić *et al.*, 2009), confirming that this cultivar was not widespread in Dalmatia in the 19th and 20th century (Savić, 2003). Stojanović (1929) writes that the best vineyards in Montenegro are in the Skadar lake region (Crmnica), which is famous for its red wines that can reach a high quality, while the wines produced along the Montenegrin seacoast are similar to those from Dalmatia. This author states that Plavac mali, Dobričić and some others are the main cultivars grown in Dalmatia, whereas he does not mention Kratošija.

Ampelographic descriptions are available for all Kratošija accessions listed in Table 1 and grown at the Experimental Estate of the Biotechnical Institute in Podgorica - Ljeskopolje (Maraš, 2000). Some examples of ampelographic variability in Kratošija accessions are reported in Table 3 and Figure 1. The name of each Kratošija biotype is in connection with some of its particular characteristics. In particular, these biotypes showed a wide range of variability in grape yield, mainly dependent on bud fertility rather than cluster weight. Cluster weight was highly variable among accessions and correlated with bunch morphology; these traits showed to be stable within each biotype (Maraš, 2000). One mother vine was selected per biotype for the sanitary check-up and all showed the presence of viruses. Furthermore, additional investigation of the sanitary status of newly selected vines of Kratošija from all viticultural regions of Montenegro showed a high level of viral infection (Maraš *et al.*, 2014).

By putting together different pieces of information from the literature cited, and given the results of our study, we can conclude that Kratošija with all its biotypes has been grown in Montenegro for centuries. Given its presence in all the viticultural regions of this little country, the wide variability of the population, the impressive high number of different names, the close genetic relationship with Vranac, and its current presence (survival) in viticultural areas in Montenegro, we suggest that this area be considered a likely region for the origin and spreading centre of this cultivar. Therefore, the place of origin of this cultivar should be moved further south than the

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Table 3 - Examples of ampelographic variability of some Kratošija accessions (Maraš, 2000) adapted to the 2nd Edition of the OIV descriptor list for grape varieties and *Vitis* species (id number in parenthesis refers to table 1)*

Accession name	OIV CODES																				
	3	**4**	**16**	**65**	**68**	**76**	**79**	**84**	**85**	**151**	**202**	**206**	**203**	**204**	**220**	**223**	**225**	**231**	**236**	**241**	**235**
Bikača (id 1)	3	7	1	7	3	2	9	5	7	3	**5**	1	**3**	5	5	2	6	3	1	3	1
Crni krstač (id 6)	3	7	1	7	**4**	2	**7**	5	7	3	7	1	5	5	5	2	6	3	1	3	1
Kratošija (id 16)	3	7	1	7	**4**	2	**7**	5	7	3	7	1	5	5	5	2	6	3	1	3	1
Kratošija ili Vran (id 20)	3	7	1	7	3	2	9	5	7	3	7	1	5	5	5	2	6	3	1	3	1
Kratošija mala (id 21)	3	7	1	7	**4**	2	**7**	5	7	3	**5**	1	5	**9**	5	2	6	3	1	3	1
Kratošija sa dub.ure. (id 9)	3	7	1	7	3	2	9	5	**9**	3	7	1	5	5	5	2	6	3	1	3	1
Kratošija srednja (id 19)	3	7	1	7	**4**	2	**7**	5	7	3	**5**	1	5	5	5	2	6	3	1	3	1
Ljutica (id 29)	3	7	1	7	**4**	2	**7**	5	7	3	7	1	5	5	5	2	6	3	1	3	1
Rehuljača (id 37)	3	7	1	**5**	3	2	9	5	7	3	7	1	**3**	**3**	5	2	6	3	1	3	1
Srednji vranac (id 38)	3	7	1	7	3	2	9	5	7	3	7	1	5	5	5	2	6	3	1	3	1
Velji vranac (id 42)	3	7	1	7	3	2	9	5	7	3	7	1	5	5	5	2	6	3	1	3	1
Vranac 9 (id 44)	3	7	1	7	3	2	9	5	7	3	7	1	5	5	5	2	6	3	1	3	1
Vran (id 43)	3	7	1	7	3	2	9	5	7	3	7	1	5	**9**	5	2	6	3	1	3	1
Vrančić (id 65)	3	7	1	7	3	2	9	5	**9**	3	7	1	5	5	5	2	6	3	1	3	1
Vrančina (id 66)	**1**	7	1	7	3	2	**7**	5	7	3	7	1	5	5	5	2	6	3	1	3	1

*traits differing in the level of expression compared to the population are in bold.

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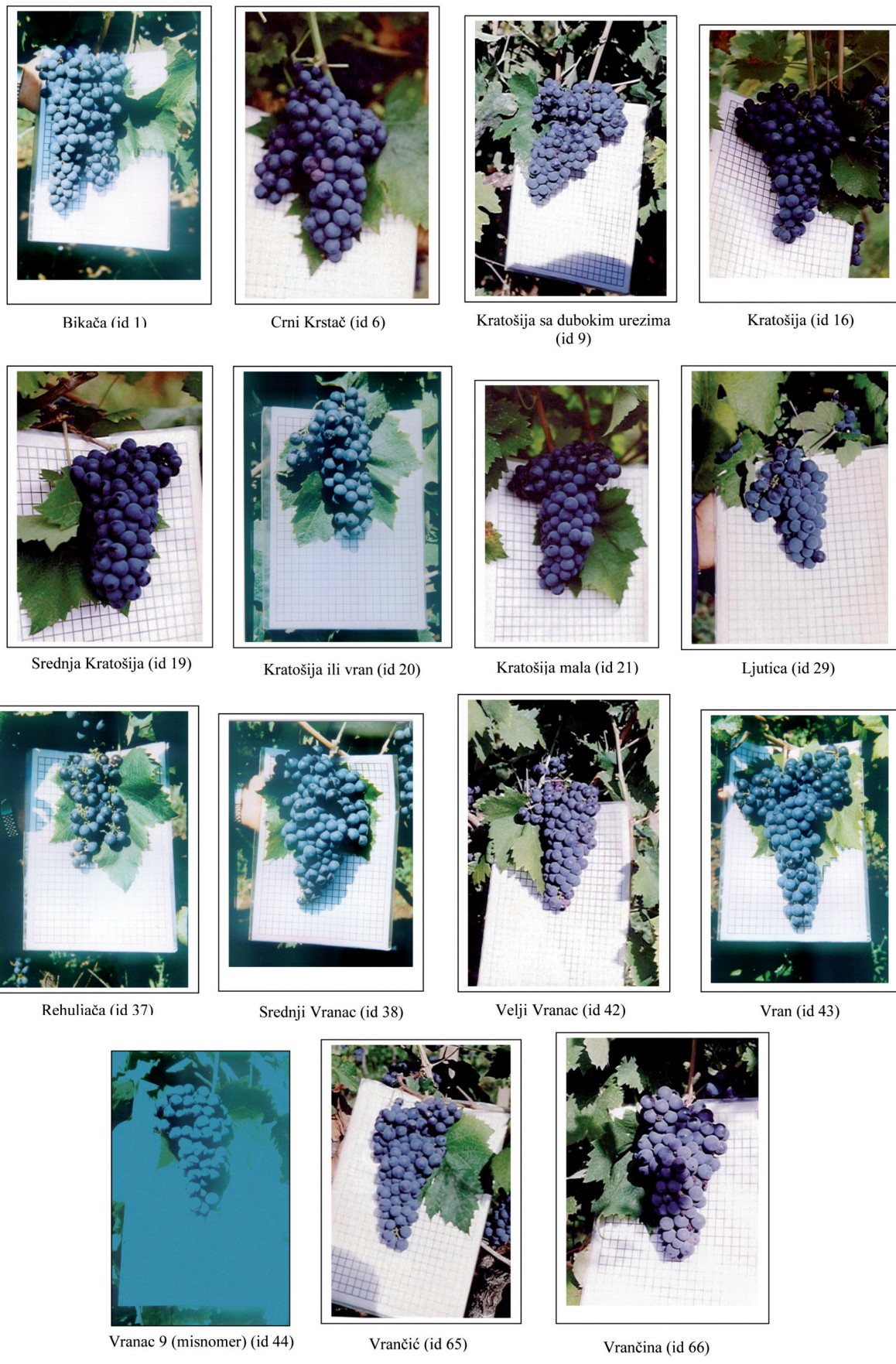


Figure 1 - Examples of cluster variability among Kratošija biotypes. In brackets the reference id number referred to table 1.

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Dalmatian coast, unlike what has been previously suggested by Maletic *et al.* (2004).

The SSR profile of Muškaćela accessions matches that of Muscat blanc à petits grains. This outcome adds a new synonym to the long list of this very ancient cultivar, largely spread all over the viticultural areas in Europe and recently showed to be the main ancestor of the Muscat family (Cipriani *et al.*, 2010). Trojka accessions have the same profile as Muscat rouge de Madere (*alias* Moscato violetto), another important member of the Muscat family (Di Vecchi-Staraz *et al.*, 2007; Lacombe *et al.*, 2013), but the berry colour is different (it is white in our analyzed Trojka cultivar). Muscat rouge de Madere is a progeny of Muscat blanc à petits grains and Sciaccarello (Lacombe *et al.*, 2013) and has little importance. Supposed to be native to Portugal, it does not have any agronomic relevance. Actually, it is not included in any official list of grapevine cultivars authorized for cultivation in 24 national catalogues (Lacombe *et al.*, 2011). It is considered a grape for enthusiasts (Galet, 2000) and is grown mostly in germplasm repositories. That is why it was a surprise to find it among Montenegrin cultivated grapevines. Plavina is a progeny of Kratošija and Verdeca (Cipriani *et al.*, 2010) and is an important cultivar in the sub region of North Dalmatia. All mother plants chosen for clonal selection of Vranac (NCV 62/13; NCV 14/39; NCV 15/13; NCV 62/7; NCV 62/8) showed to be correctly identified.

Although in literature Bioka is considered as a synonym of Krstač in Crmnica (Ulićević, 1959), SSR data showed that it has a different molecular profile. Therefore, Bioka is another cultivar. We have discovered that Bioka shares the same genotype as the Italian Francavidda, a cultivar registered in the Italian Catalogue and grown in Apulia, the Italian region facing Montenegro on the other side of the Adriatic Sea (http://catalogoviti.politicheagricole.it). Moreover, Bioka has the same SSR profile as the Croatian Zlatarica Vrgorska (Zdunić *et al.*, 2013). Therefore, these three cultivars represent a new case of previously unknown synonyms grown in different countries facing the shores of the Adriatic Sea (Dalmatia and Apulia).

Comparing our data with the European *Vitis* database (http://www.eu-vitis.de/index.php), we found one match at all seven common SSR loci between the Montenegrin Kadarun and a Kadarun accession from Croatia (HRV041-SA#11-95) lacking the true-to-type validation. In the *Vitis* International Variety Catalogue (VIVC, http://www.vivc.de), Kadarun is reported as a Turkish cultivar.

The red berry Razaklija accession from the Skadar Lake region (Doljani), a known synonym of the Serbian Drenak crven, matches the SSR profile of Crven Drenok (Vitis-WBC, Western-Balkans *Vitis* Database, http://vitis.atcglabs.com). Table grapes in Montenegro only accounts for 5 %, of which 95 % is the Razaklija cultivar (Drenak crven) from both the Skadar Lake and the seacoast region (Ulićević, 1959). Many authors from Yugoslavia consider that Razaklija originates from Asia Minor (Ulićević, 1966). It is not known how and when it arrived in Montenegro, Macedonia and other countries and how it spread.

Crna Loza, Čubrica, Krstač, Lisičina, Razaklija crna and Žižak show unique SSR profiles. Crna Loza was considered as a Kratošija synonym, but our analysis shows a different SSR profile for this cultivar. Based on SSR allele sharing at all analyzed loci, Crna Loza could be another offspring of Kratošija, but further research should be done to confirm this hypothesis.

Ulićević (1959) states that Čubrica is a cultivar for red wine production and is represented in very small percentage in the vineyards of the Podgorica sub region (Doljani and Kuci). The sample of Čubrica from Kuci has a DNA profile confirming that it is an independent cultivar.

Ulićević (1959) writes that in the Skadar Lake region, in the sub region of Podgorica (Beri and Doljani), Krstač was dominant among the white grapevine cultivars used for white wine production, produced in small amount in that period. In the sub region of Podgorica, it represents only 10 % of the vineyard area, mostly in Beri and Doljani, and very little in the Ljesanska district. Krstač is believed to be autochthonous of Montenegro and probably originated in Beri (near Podgorica); its name comes from the look and shape of the bunch that resembles a cross (Viala and Vermorel, 1910; Vujović, 1956). Žižak (or Žižak bijeli after Bulić, 1949) is considered another autochthonous cultivar of Montenegro and its origin is unknown. Individual specimens can be found nearby Podgorica, but it is mostly grown on the Montenegrin seacoast (Boko-Kotorski sub region), where it is used for dessert wine (Ulićević, 1959).

About Lisičina, Stojanović (1929) reports the use of this cultivar for white wine production in Montenegro. Bulić (1949) and Ulićević (1959) found Lisičina (synonyms are Lisica, Ružica, Sjemerava, Sjeruša) in Montenegro around Bar, Ulcinj, Podgorica and Virpazar (Crmnica). The VIVC catalogue reports that Lisičina is a synonym of the Croatian Lisica. According to Nastev (1967), Lisičina is a wrong synonym of the Plovdina (Pamid) cultivar.

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Accordingly, the SSR profile of our Lisičina sample is different from that of the Bulgarian Pamid (Hvarleva *et al.*, 2004 ; Dzhambazova *et al.*, 2009).

Razaklija crna (black), having a dark blue coloured skin, was found only in Kuci (sub region of Podgorica) in old vineyards and under this name. It has to be noted that this name indicates a table grape cultivar originated by crossing Razaklija Rossa and Vranac (performed by Ulićević M. at the Biotechnical Institute in Podgorica). Based on SSR allele sharing at all analyzed loci, Razaklija crna could really be a progeny of Drenak Crven, but our SSR data rule out Vranac. Further data are required to validate this partial pedigree.

CONCLUSION

Seventy accessions belonging to the Montenegrin grapevine germplasm were analyzed using SSR markers. Fourteen genotypes have been found, three of them being native to Montenegro (i.e., Vranac, Krstač and Žižak). Kratošija has been recently recognized as an international cultivar, being a synonym of Italian Primitivo, Californian Zinfandel and Croatian Crljenak Kaštelanski, whose origin has been widely investigated. Given the large number of different names used in Montenegro for Kratošija and the wide morphological variability of Kratošija biotypes, we propose here, for the first time, Montenegro as the best candidate country for the origin and spreading centre of this group of synonyms. Crna Loza, Čubrica, Lisičina and Razaklija crna are cultivars likely native to Montenegro. Muškaćela and Trojka, considered autochthonous of Montenegro, are clearly of different origin. We discovered that Montenegrin Bioka, Italian Francavidda and Croatian Zlatarica Vrgorska represent a new group of synonyms.

Overall, our genotyping study gave us the opportunity to check the available information based on ampelographic descriptions and comparisons, providing a more stringent and sometimes new perspective on the origins and relatedness of grapevine cultivars in Montenegro.

Further analyses should be done in order to clarify the genetic relationships among Montenegrin grapevine cultivars as well as the degree of relatedness and origin of commercially important Montenegrin cultivars.

The sharing of common cultivars among neighbouring countries was expected as a natural consequence of easily vegetative propagation of grapevine, but only recently we have started to get accurate information on their identity and the present research contributes to shed some light on this topic.

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