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Ampelographic and genetic characterization of Croatian grapevine varieties

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Summary

Before the Phylloxera (*Daktulosphaira vitifoliae*) crisis in Croatia more than 400 grape varieties were under cultivation. Today their number is drastically decreased. Recently, many efforts in the preservation of grapevine biodiversity were undertaken: detailed inventory of varieties in different wine-growing regions, establishing of national and regional collections and their systematic ampelographic and genetic characterisation. This paper shows results of multiannual ampelographic and genetic research concerning Croatian grapevines considered as autochthonous varieties. After identification, many synonyms and homonyms were detected and unique genotypes were selected. Basic data of them are shown: description (according to OIV descriptors) and genetic profiles (9 SSR loci), growing area and status of vulnerability. Also, genetic relationship based on the shared allele distance is computed from SSR data. Results show certain level of similarity among varieties, and classify Croatia as important gene-pool in Europe. Some additional accessions which were detected and introduced into collections recently are still being investigated and characterized so that the here presented list of Croatian native grapevine cultivars is not final.

Key words: grapevine germplasm; preservation; Croatian autochthonous cultivars; SSR; characterization; ampelography; documentation.

Introduction

First evidence of viticulture in Croatia dates back to the bronze age, around 1800 BC (BATOVIĆ and KUKOČ 1987). At the end of the 19th and the beginning of the 20th century, Croatia was a significant European vine growing country. At that time there were about 200,000 hectares under vineyards, with 90,000 ha in the Dalmatia region alone and presently only ca 25,000 ha of vineyards remained.

The oldest ampelographic studies of Croatian genotypes were published in the beginning of the 19th century by RITTER (1821), STANKOVICH (1824), TRUMMER (1841) and especially by the end of 19th century by STRAZIMIR (1876) and GOETHE (1887). BULIĆ (1949) completed the first book

of ampelography and documented almost 200 grapevine cultivars grown in Dalmatia during the period 1887–1925. Besides favorable climatic conditions, a turbulent past and intense trade and exchange with other countries where grapes were grown increased the number of cultivars. It is probable that some of them were developed in Croatia, while others were introduced a long time before. Unfortunately, many cultivars got lost at the beginning of the last century due to vineyard destruction caused by introduced fungal diseases and pests (e.g. *Plasmopara viticola*, *Erysiphe necator*, *Daktulosphaira vitifoliae*). Further reasons for drastic erosion of native cultivars were the growers' demands for high yields and the introduction of world-wide known cultivars (e.g. 'Chardonnay', 'Riesling', 'Cabernet Sauvignon' and 'Merlot'). Very likely many of the valuable autochthonous cultivars are now extinct and still existing neglected germplasm is subject to continuing eradication. On the other hand native cultivars are still playing an important role in Croatian wine production. Moreover, at present the most awarded and appreciated Croatian varietal wines on the market derive from native cultivars such as 'Plavac mali', 'Malvazija istarska', 'Pošip' and 'Žlahtina'.

A project of ampelographic and genetic identification of endangered native grapevine cultivars was launched in 1998 at the Faculty of Agriculture, University of Zagreb. Up to this date, several national and international projects focused on preservation, evaluation and revitalization of autochthonous grapevine cultivars. In 1998, Croatia became an associated partner of the GENRES#081 project. Since that time, we followed the project's harmonized methods of descriptions for cultivar identification and we contributed in establishing a common European network for preservation of genetic resources of *Vitis vinifera* which is continuously working since that time through the different projects (GrapeGen 06, COST, SEERA.net).

Native cultivars found in old vineyards turned out to be highly virus infected (KAROGLAN KONTIĆ *et al.* 2009, POLJUHA *et al.* 2010) which is also the case for the material maintained in collections (VONČINA *et al.* 2011). High level of intravarietal variability was also detected for most of the important native cultivars (PREINER *et al.* 2012, ZDUNIĆ *et al.* 2012). According to these results sanitary and clonal selection of the most important native cultivars was started.

Evaluation of quality traits of native cultivars also started. A first report on organic acid profiles of most im-

portant native cultivars showed high level of variability among them (PREINER *et al.* 2014). Ongoing research is focused on polyphenols and aromatic profiles of native cultivars. High level of variability of ampelometric parameters among native cultivars was also detected and a model for cultivar identification using these parameters was developed (PREINER *et al.* 2014).

Recent genetic research resulted in revealing synonyms and homonyms, as well as genetic relationship within Croatian native cultivars and with cultivars from other regions (MALETIĆ *et al.* 2014, MALETIĆ *et al.* 2004, ŽULJ MIHALJEVIĆ *et al.* 2013).

Several years of intensive biodiversity research resulted in the establishment of a grapevine repository of Croatian native varieties. Besides the National collection there are two regional germplasm repositories in Split and Poreč, as well as several ampelographic back-up collections in European countries, serving safeguard conservation of local varieties. In this way most of the native germplasm is duplicated.

The main goals of this research are: (a) to determine the number of Croatian native grapevine cultivars based on inventarisation of viticultural areas; (b) to perform detailed ampelographic characterization using OIV descriptors and (c) to perform the genetic characterization of Croatian native grapevine cultivars using SSR markers.

Material and Methods

In Croatia, inventarisation of grapevine cultivars started in 1998. The aim was discovery and identification of native cultivars. At the beginning, identification was based on old literature providing the basic information on historical growing areas of some native cultivars. Vines of potentially native cultivars were selected and marked. From the marked plants propagating material was taken for the establishment of the national grapevine collection of Croatia, located at the Experimental Station Jazbina, at Faculty of Agriculture, University of Zagreb. According to their extinction risk in Croatia, varieties are classified in following International Union for Conservation of Nature Red List Categories (IUCN 2001): critically endangered (CR), endangered (EN), vulnerable (VU), near threatened (NT) and least concern (LC).

In this process, 140 accessions were introduced into this collection and used in this research. SSR genotyping was performed using a total of nine markers. Six SSR markers (VrZAG62, VrZAG79, VVMD5, VVMD7, VVMD27, VVS2) recommended by OIV and three additional ones (VVMD25, VVMD28, VVMD32) selected during the GrapeGen06 project. PCR amplification, electrophoretic separation and sizing were performed as described in ŽULJ MIHALJEVIĆ *et al.* (2013).

Morphological description of collected accessions was performed using 47 OIV descriptors for grapevine cultivars and *Vitis* species (OIV 2009) that were selected within the GrapeGen06 project. Recorded data were uploaded into the European *Vitis* Database (MAUL *et al.* 2012). Ten chosen

OIV descriptors will be used to present basic information on Croatian native grapevine cultivars: OIV151 (flower sexual organs), OIV202 (bunch length), OIV204 (bunch density), OIV208 (bunch shape), OIV220 (berry length), OIV223 (berry shape), OIV225 (berry skin color), OIV236 (berry particular flavor), OIV505 (sugar content of must) and OIV506 (total acidity of must). Complete OIV description and SSR profiles of the cultivars are available in the European *Vitis* Database (MAUL *et al.* 2012).

Results and Discussion

Based on results of ampelographic characterization and genetic characterization, out of the 140 accessions analyzed, the list of 103 unique genotypes (Croatian native grapevine cultivars) is defined together with their synonyms and growing area/vulnerability evaluation (Table). In this table, information on the collection where it is maintained is given for every cultivar. Some additional accessions which were detected and introduced into collections recently are still being investigated and characterized so that the here presented cultivar number is not final.

Based on presented information on status of Croatian native grapevine cultivars we can conclude that more than 50 % of cultivars are critically endangered (40), endangered (7) or vulnerable (11). Only two cultivars ('Plavac mali' and 'Malvazija istarska') are to be considered stable and safe (least concern), while rest of them (48) are considered near threatened.

SSR profiles of the Croatian native cultivars are uploaded in European *Vitis* Database (MAUL *et al.* 2012) and are available upon request. Among the 103 unique genotypes of Croatian native cultivars analyzed with 9 SSR markers, a total of 89 alleles were detected with a mean value of 9.9. Locus VVMD27 had the lowest number of alleles (7), while locus VVMD28 was the most polymorphic with 13 alleles. Average observed heterozygosity was 0.824 and higher than expected (0.796). Polymorphism information content varied between 0.688 (VVMD25) and 0.860 (VVMD28) with mean value of 0.763. These values are in accordance with similar studies (ŽULJ MIHALJEVIĆ *et al.* 2013) that analyzed cultivars from Croatia and neighboring countries. Studies with much larger sets of diverse cultivars from different geographic regions, like LAUCOU *et al.* (2011) detected higher numbers of alleles.

Comparison of Croatian cultivars SSR profiles with other cultivars fingerprints from the European *Vitis* Database confirmed that the majority are unique genotypes and exist only in Croatia. Since most of them are duplicated in grapevine germplasm collections throughout Croatia they are considered as "saved". However, further research is needed to identify cultivars with economic potential as well as those with valuable traits that can be used in breeding. Ongoing genetic studies with increased number of SSR loci (> 30) as well as the use of SNP markers will allow a more detailed analysis of genetic structure and relatedness of Croatian native cultivars. The results of ampelographic characterization of Croatian native cultivars are showing

Table

List of Croatian native grapevine cultivars, their synonyms, information on their growing area and extinction risk

No	Prime name	Berry colour ¹	Main and new synonyms	Extinction risk ²	Preserved in collection ³	Growing region ⁴	Total area (ha) ⁴
1	Babica	N		NT	AF, IJK	DAL	18.55
2	Babić	N	Rogoznička, Šibenčanac, Babicaplosnata	NT	AF, IJK	DAL	373.1
3	Bak	N	Siložder	CR	AF, IJK	DAL	
4	Bilan	B	Vitovskagrganja, MalvasiaFurmhann	CR	AF	IHP	0.06
5	Bogdanuša	B	Bogdanjuša, Vrbanjka	NT	AF, IJK	DAL	50.79
6	Brajda velika	N	Sušac, Sušić, Bašćan, Starabrajda	VU	AF, IJK	IHP	1.22
7	Brajdica bijela	B		VU	AF	IHP	0.5
8	Bratkovina bijela	B	Pošipica, Brakovina	VU	AF, IJK	DAL	1.53
9	Bratkovina crvena	Rg	Pošipiacrvena, Lelekuša	CR	AF	DAL	0.04
10	Bumba	B	Medna, Zložder	NT	AF	DAL	9.14
11	Cetinka	B	Blatinka, Blajka, Petovka	NT	AF, IJK	DAL	35.74
12	Cibib	B		CR	AF	DAL	
13	Cipar	Rs	Grec rouge, Kalebstraube	CR	AF, IJK	DAL	
14	Crljenak kaštelanski	N	Primitivo, Tribidrag, Zinfandel, Kratošija, Pribidrag	NT	AF, IJK	DAL	74.11
15	Crljenak viški	N	Crljenakrni	CR	AF, IJK	DAL	
16	Crnka	N		CR	AF, IJK	DAL	
17	Debejan crni	N		VU	AF	IHP	0.93
18	Debit	B	Puljižanac	NT	AF, IJK	DAL	413.2
19	Dišeća ranina	B	Dišećabelina, Urbanitraube	NT	AF	SJZ	2.36
20	Divljaka bijela	N		CR	AF	DAL	
21	Dobričić	N	Šoltanac, Slatinjanac	NT	AF, IJK	DAL	7.17
22	Dolcin	B		CR	AF, IPT	IHP	
23	Draganela	B	Martinšćica, Belej	CR	AF	IHP	0.07
24	Drnekuša (vela)	N	Darnekuša, Glavanjuša	NT	AF, IJK	DAL	3.77
25	Dugovrst	B	Dugolist, Dugoviska	VU	AF	DAL	1.12
26	Frmentun	B		CR	AF, IJK	DAL	
27	Galac	N	Gavran	CR	AF	DAL	
28	Garganja	B	Opačevina	CR	AF, IPT	IHP	
29	Gegić	B	Paška, Belidebejan	NT	AF, IJK	DAL	20.22
30	Glavinuša	N	Okatac	NT	AF, IJK	DAL	5.44
31	Grk	B	Lumbarajskogark	NT	AF, IJK	DAL	15.5
32	Gustopupica	N	Čestopupica	CR	AF, IJK	DAL	
33	Hrvatica	N	Crevatizza, Kamenina	NT	IPT	IHP	3
34	Jarbola	B		VU	AF	IHP	0.43
35	Kadarka	N	Skadarka, Braničevka	NT	AF	SPD	6.11
36	Kadarun	N		NT	AF	DAL	8.11
37	Klešćec	B		VU	AF	SJZ	0.69
38	Kozjak bijeli	B	Kozjesise, Geisdutteweisse, Coarna alba	CR	AF	SJZ	
39	Kraljevina	Rs	Imbrina, Portugieserroter	NT	AF	SJZ	272.2
40	Krivalja crvena	N	Krivajacrvena, Cornichon violet	CR	AF, IJK	DAL	
41	Krstićevica	B	Karstićevica	CR	AF, IJK	DAL	0.05
42	Kujundžuša	B	Žutac, Tvrdac	NT	AF, IJK	DAL	234.2
43	Kurtelaška	B	Kortolaška, Kurtelaška, Kurtolaška	NT	AF	DAL	2.64
44	Lasina	N	Šljiva, Zlaorina, Vlašicrljenak	NT	AF, IJK	DAL	16.05
45	Ljutun	N	Ljutacrni	VU	AF, IJK	DAL	0.62
46	Magrovina	N		CR	AF	IHP	
47	Malvasija dubrovačka	B	Malvasia di Lipari, Malvasia di Sardegna, Malvasia di Bosa, Malvasia de Sitges, Malvasia de la Palma	NT	AF, IJK	DAL	30.73
48	Malvazija istarska	B	Očenaš, Vrbić	LC	AF, IJK, IPT	IHP	1778
49	Maraština	B	Rukatac, Malvasia binca lunga, Malvasia del Chianti, Pavlos	NT	AF, IJK	DAL	303.1
50	Mekuja	B	Mekulja	VU	AF	DAL	0.81
51	Mijajuša	N	Boglička, Xeromachairouda, XerichiKokkino	CR	AF, IJK	DAL	
52	Mirkovača	B		CR	AF	SJZ	
53	Mladenka	B	Mladinka	NT	AF, IJK	DAL	8.37
54	Moslavac	B	Šipon, Pušipel, Moslgerber, Furmintfeher	NT	AF	SJZ	160.8

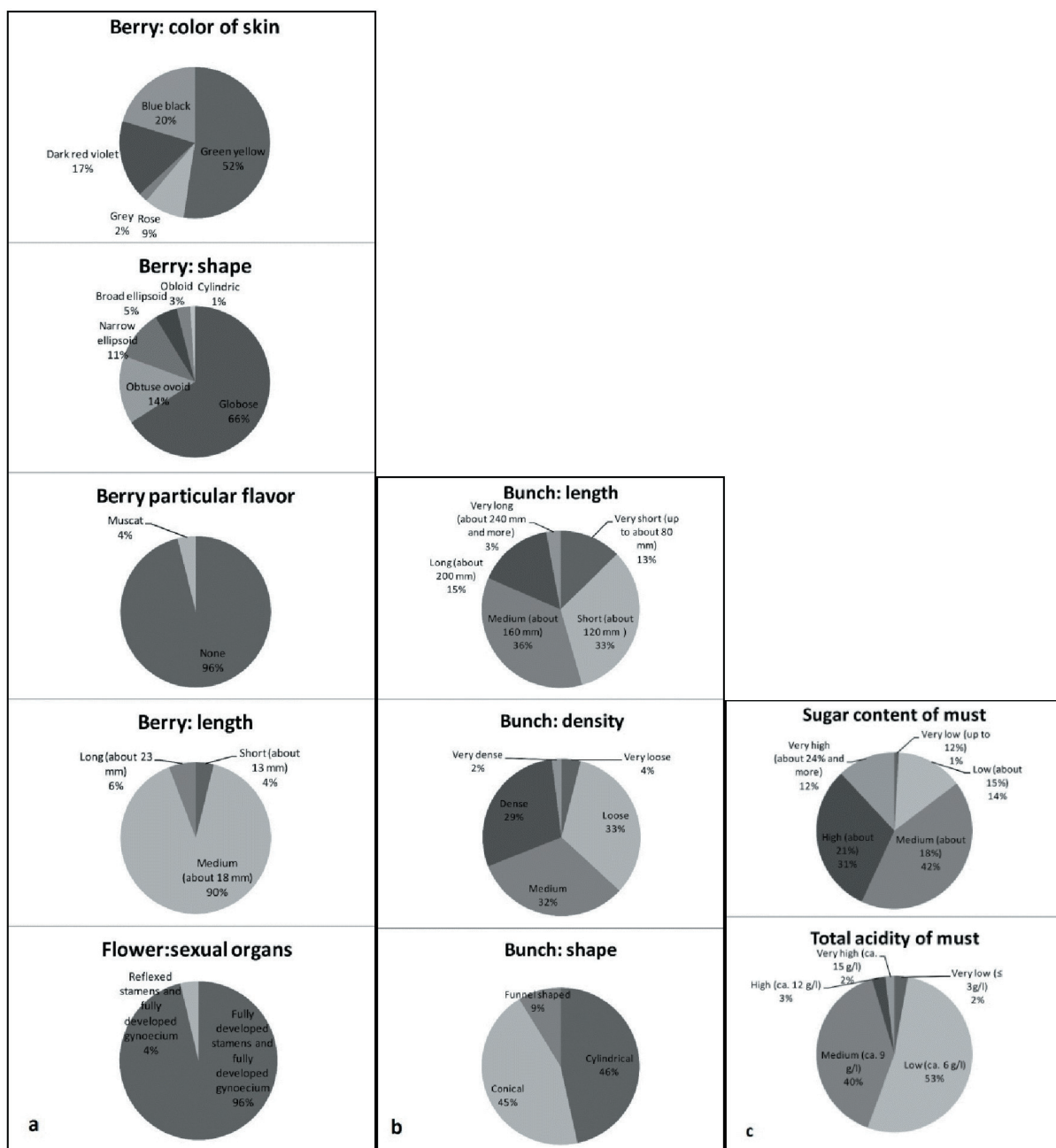
Table, continued

No	Prime name	Berry colour ¹	Main and new synonyms	Extinction risk ²	Preserved in collection ³	Growing region ⁴	Total area (ha) ⁴
55	Muškat ruža	N	Muškatružaporečki, Rosenmuskateller, Moscato rosa	NT	AF, IPT	IHP	8.37
56	Ninčuša	N	Linčuša	NT	AF, IJK	DAL	19.5
57	Okatica bijela	B		NT	IJK	DAL	4.17
58	Ošljevin	B		CR	AF	IHP	
59	Palagružanka bijela	B	Nastriženica, Palagružonka	EN	AF, IJK	DAL	0.12
60	Palaruša	B	Podričuša	NT	AF, IJK	DAL	0.1
61	Plavica	Rg	Plavackrčki, Plavacveli	CR	AF	IHP	
62	Plavac mali crni	N	Crljenakmali, Pagadebitcrni	LC	AF, IJK	DAL	1697
62a	Plavac mali sivi	G		VU	AF, IJK	DAL	1.19
63	Plavčina	Rg		EN	AF	IHP	0.13
64	Plavec žuti	B		NT	AF	SJZ	14.33
65	Plavina	N	Plavka, Brajdica, Marasovka	NT	AF, IJK	DAL	673.8
66	Pljuskavac	N	Bljuzgavac, Blanc blauer, Kesnačrnina	CR	AF	DAL, SJZ	
67	Pošip bijeli	B		NT	AF, IJK	DAL	283.3
68	Pošip crni	Rg	Razaklija, Šljiva	EN	AF, IJK	DAL	0.36
69	Prč	B		NT	AF, IJK	DAL	7.54
70	Pršljivka	B		CR	AF	DAL	
71	Ranfol	B	Štajerskabelina, Štajerka, Svetljak	NT	AF	SJZ	135.2
72	Rušljin	Rg		CR	AF	IHP	0.01
73	Ruža bijela	B		CR	AF	DAL	
74	Silbijanac	B	Ranacsilbanjski	CR	AF, IJK	DAL	
75	Sušćan		Sansigot	NT	AF	IHP	3.76
76	Surina	Rs		CR	IPT	IHP	
77	Sverdlovina crna		Galicacrna	EN	AF, IJK	DAL	0.38
78	Smudna belina	B		CR	AF	SJZ	
79	Sokol		Lugliengabianca, Seidentraube	CR	AF	SJZ	
80	Stara hrvatska belina	B	Gouais b., WeisserHeunisch	CR	AF	SJZ	
81	Šemperinka	N	Šeperinka	CR	AF	DAL	
82	Šipelj	B		NT	AF	SJZ	12.81
83	Škrljet	B	Ovnekžuti	NT	AF	SJZ	69.9
84	Teran	N	Terin, Istranin, Istrijanac, Terano	NT	AF, IPT	IHP	242.8
85	Topol	B	Beretinjokbijeli, Tikvar	CR	AF, IJK	DAL	
86	Trbljan	B	Dobrogostina, Kuč, Tarpinka, Filipić	NT	AF	DAL	258.9
87	Trišnjavica	Rs	Šaricatrišnjavica	CR	AF, IJK	DAL	
88	Trnjak crni	N	Trnjak, Rudežuša	NT	AF	DAL	21.1
89	Trojiščina crvena	Rs		EN	AF	IHP	0.22
90	Vela pergola	B		CR	IPT	IHP	
91	Vlaška	B	Žutulja, Tanetovaloza	NT	AF, IJK	DAL	9.84
92	Volarovo	B		EN	AF	IHP	0.33
93	Volovina crvena	G	Muscat rouge de Madère	CR		SJZ	
94	Vrškajica	B	Vrškarića	CR	IJK	DAL	
95	Vugava bijela	B	Viškulja, Bugava, Ugava,	NT	IJK	DAL	41.49
96	Vugava crvena omiška	Rg		CR	IJK	DAL	
97	Zadarka	N		EN	AF, IJK	DAL	0.23
98	Zlatarica blatska	B		VU	AF, IJK	DAL	0.56
99	Zlatarica vrgorska	B	Dračkinja, Zlatarica	NT	AF, IJK	DAL	20.03
100	Žilavka	B	Žilavkamostarska	NT	AF, IJK	DAL	9.91
101	Začreška belina	B	Svetokriškabelina	CR	AF	SJZ	
102	Žlahtina	B		NT	AF	IHP	152
103	Žumić	B		CR	AF	IHP	0.04

¹) Berry color: N – blue black and dark red violet, B – green-yellow, Rs – rose, Rg – grey; ²) Extinction risk in Croatia, varieties are classified in following IUCN Red List Categories (IUCN, 2001): CR - critically endangered, EN – endangered, VU – vulnerable, NT - nearly threatened, LC - least concern; ³) AF-Faculty of Agriculture Zagreb, IJK-Institute for Adriatic Crops and Karst Reclamation Split, IPT- Institute of Agriculture and Tourism Poreč, ⁴) SJZ-Northwest Croatia, SPD-Slavonia and Danube Region, IHP-Istria and North Adriatic Coast, DAL-Dalmatia.

the high level of variability for all of the 47 descriptors. To present some basic information on characteristics of this cultivars 10 traits are chosen and presented in Figure a (berry characteristics), b (bunch characteristics) and c (ba-

sic quality characteristics). Within 103 cultivars, dominant color of berry skin is green-yellow, which is determined for 52 % of cultivars, and rests of them are having some level of anthocyanin coloration present in the berry skin (from



Figure, a: berry characteristics, b: bunch characteristics and c: basic quality characteristics of Croatian native grapevine cultivars.

gray to blue-black). Most common berry shape is globose (66%), followed by obtuse ovoid (14%), and narrow ellipsoid (11%) shapes. Medium size berries are the most common and determined for 90% of the cultivars. This can be explained with the fact that most of Croatian native cultivars are used for wine production, some are used for combined wine and table grape production, and only few are strict table grapes. Most of the cultivars (96%) have no particular flavor, while the rest of them exhibit muscat flavor. Morphologically and functionally hermaphrodite flower is present at 96% of the cultivars, while others have female type of flower (reflexed stamens). This has a complex impact on their production characteristics, especially yield and quality (PREINER *et al.* 2012). Basic bunch characteristics of native cultivars are presented in Figure b. Medium and short bunches are the most common type

within Croatian cultivars, followed by cultivars with long, and very short bunches. This can also be explained with the fact that most of the Croatian native cultivars are wine grapes. Native cultivars are represented with the same portion in three bunch density classes: dense, medium and loose, while the dominant shapes of the bunches are cylindrical and conical. Basic quality parameters (must sugar content and total acidity) of Croatian native grapevine cultivars are presented in Figure c. Most of the cultivars are characterized with medium and high level of must sugar content and with the group of cultivars with very high sugar content they represent 85% of all native cultivars. This fact shows that most of the native cultivars are having good quality potential for wine production. Total acidity of the must of more than 90% of the native cultivars is low and medium.

Conclusion

Based on the results presented in this paper and considering the small viticultural area it can be concluded that Croatia has a relatively high number of native cultivars. On the other hand, comparison of the number of cultivars which are preserved today with the historical data we can also conclude that many of the native cultivars were lost during the 20th century, a phenomenon stated in all European wine-growing countries, as the importance of that cultural heritage was not recognized at that time. This is also evident based on the fact that more than 50 % of the still existing native cultivars are endangered because of the few plants which remained in old vineyards, a high level of virus infections, high intravarietal variability and lack of quality propagation material.

The preserved native cultivars are presenting a good base for future development of Croatian viticulture. Because of the high genetic variability and some valuable traits Croatian native cultivars could be of interest to other countries for production as well as for breeding.

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