

## Molecular characterization of old local grapevine varieties from South East European countries

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### Summary

South East European (SEE) viticulture partially relies on native grapevine varieties, previously scarcely described. In order to characterize old local grapevine varieties and assess the level of synonymy and genetic diversity from SEE countries, we described and genotyped 122 accessions from Albania, Federation of Bosnia and Herzegovina (B&H), Croatia, Macedonia, Moldova, Montenegro, Republika Srpska (Bosnia and Herzegovina) and Romania on nine most commonly used microsatellite loci. As a result of the study a total of 86 different genotypes were identified. All loci were very polymorphic and a total of 96 alleles were detected, ranging from 8 to 14 alleles per locus, with an average allele number of 10.67. Overall observed heterozygosity was 0.759 and slightly lower than expected (0.789) while gene diversity per locus varied between 0.600 (VVMD27) and 0.906 (VVMD28). Eleven cases of synonymy and three of homonymy have been recorded for samples harvested from different countries. Cultivars with identical genotypes were mostly detected between neighboring countries. No clear differentiation between countries was detected although several specific alleles were detected. The integration of the obtained genetic data with ampelographic ones is very important for accurate identification of the SEE cultivars and provides a significant tool in cultivar preservation and utilization.

**Key words:** *Vitis vinifera*, microsatellites, genotyping, South East European germplasm.

### Introduction

The viticulture of South East Europe (SEE) is to some extent "personalized" due to the large number of autochthonous (unique) varieties. Wine production has a significant impact on the economy of the partners countries involved in this study, but it is also tightly connected with the history and tradition of each country. This richness of

native varieties which results in unique wines has great potential for the future of winemaking in Europe. On the other hand, it requires a lot of work in order to properly maintain germplasm, perform clonal selection and ensure high quality plant material production.

Vegetative propagation enabled conservation of cultivars over a long period. At the end of the 19<sup>th</sup> century, pests and pathogens from America reached Europe (*Plasmopara viticola*, *Uncinula necator*, *Daktulosphaera vitifoliae*) resulting in devastation of many European vineyards and drastically changing the diversity of grapevine (THIS *et al.* 2006). In the 20<sup>th</sup> century, global development of the wine grape industry further restricted the varieties in cultivation and led to the wide diffusion of a small number of French cultivars (CIPRIANI *et al.* 2010). A progressive reduction of the genetic diversity of crop plants is a currently occurring phenomenon and "genetic erosion" especially affects local autochthonous varieties (EL OUALKADI *et al.* 2011). Many local grapevine varieties traditionally grown were abandoned in favor of varieties more adapted to the wine market demand and they have only recently been introduced back into cultivation, in order to locally diversify the market (CIPRIANI *et al.* 2010). Grapevine projects around the world are rescuing varieties under risk of extinction and those rescued are preserved in grapevine collections (GARCÍA-MUÑOZ *et al.* 2012). During the long period of cultivation, cultivar names were often changed because of transliteration, substitution of local name, presence of clones within cultivars, poor documentation and lack of knowledge resulting in numerous cultivars that have synonyms and homonyms within and among countries (CIPRIANI *et al.* 2010, GARCÍA-MUÑOZ *et al.* 2012). The European *Vitis* Database currently comprises 32,410 accessions from 35 grapevine repositories originating from 22 vine growing nations (MAUL *et al.* 2012). However, from the SEE region only data from Croatia and Moldova are available. Recently, the ampelographic and molecular characterization of local varieties has been done in many European countries, like Spain (SANTANA *et al.* 2008, VILANOVA *et al.* 2009, SANTANA *et al.* 2010, MARTÍN *et al.* 2011), Italy (SCHNEIDER *et al.* 2001, TORELLO MARINONI *et al.* 2009, CIPRIANI *et al.* 2010)

Portugal (LOPES *et al.* 1999), Austria (SEFC *et al.* 1998), Slovenia (ŠTAJNER *et al.* 2011). Similar characterization has also been done recently in countries of the Mediterranean basin like Turkey (ŞELLI *et al.* 2007, BOZ *et al.* 2011), Algeria (LAIADI *et al.* 2009), Tunisia (ZOGHLAMI *et al.* 2009), Morocco (EL OUALKADI *et al.* 2011) and Cyprus (HVARLEVA *et al.* 2005). In the last few years even larger studies have been performed by CIPRIANI *et al.* 2010 and LAUCOU *et al.* 2011 but still South East Europe is poorly represented.

Unfortunately, in the SEE countries the level of information on native germplasm and its present preservation status is insufficient and fragmented. In the past years, however, some research has been done on identification and evaluation of genetic diversity of autochthonous grapevine varieties (MALETIĆ *et al.* 1999, LADOUKAKIS *et al.* 2005, BENJAK *et al.* 2005, GHEORGHE *et al.* 2008, ŠTAJNER *et al.* 2009, GHETEA *et al.* 2010). Still, for many old varieties from the SEE region data and/or planting material needed for research, breeding or growing are not available and many of them have unknown genetic profiles. This information could be very important for managing genetic biodiversity as well as for elucidation of genetic relationship between varieties. Generally, most information is usually collected, described, analyzed or preserved following very different methodologies which make its exchange and use very difficult. Thus, there is a great need for regional collaboration in grapevine germplasm analysis in order to make a thorough inventory and an efficient regional plan for germplasm preservation and utilization.

As a part of a regional project (see Acknowledgements) the aim of this study was to perform ampelographic and molecular characterization of some old local grapevine varieties, traditionally grown and supposed to be native in each SEE country. Also, this paper reports an assessment of the level of genetic diversity and elucidates synonymous varieties among regions.

## Material and Methods

**Plant material and DNA extraction:** Plant tissue for DNA extraction was collected from a total of 122 grapevine accessions originating from seven South East European countries. Number of accessions per country varied between 10 (Albania) and 26 (Romania). Two entities of Bosnia and Herzegovina (Federation of B&H and Republika Srpska) participated in the SEEDNet project independently with 12 and 13 accessions, respectively (Tab. 1). Young leaves from a single vine were taken during May and June from the official germplasm collections (all the samples from Croatia, Moldova and Romania; and two samples ('Vranec' and 'Kratoshija') from Macedonia) and in all other cases from *in-situ* vineyards. Variety identification for sampling was performed by experienced ampelographers or viticulturists. DNA was extracted from 20 mg of lyophilized leaf tissue according to Qiagen DNeasy Plant Mini Kit protocol (QIAGEN, Germany).

**Microsatellite analysis:** Analysis was performed using nine microsatellite loci: VVS2 (THOMAS and SCOTT 1993), VVMD5, VVMD7, VVMD25, VVMD27,

VVMD28, VVMD32 (BOWERS *et al.* 1996, 1999), VrZAG62 and VrZAG79 (SEFC *et al.* 1999). This set of highly polymorphic markers was used by the European GrapeGen06 consortium (<http://www1.montpellier.inra.fr/grapegen06/accueil.php>) as the standard set for the screening of grapevine collections.

PCR amplifications were carried out in Veriti™ Thermal Cycler (Applied Biosystems, Foster City, California, USA). Two multiplex PCR reactions were carried out for five and three of the analyzed SSRs and a singleplex for VVMD5. The first multiplex reaction consisted of VVS2, VVMD7, VVMD27, VrZAG62, VrZAG79 loci. In the second multiplex VVMD25, VVMD28, VVMD32 were amplified.

All forward primers were labeled with 6-FAM, VIC, PET, or NED fluorescent dyes. The reactions were prepared in a final volume of 10 µL, containing 25 ng genomic DNA, 1 U Taq polymerase (Sigma-Aldrich, Germany), 0.2 mM of each dNTP, 0.2 µM of each forward and reverse primer, 2X PCR buffer, 2.5 mM of MgCl<sub>2</sub> and 1X Q solution (Qiagen, Hilden, Germany). Singleplex was performed in a final volume of 10 µL containing 25 ng genomic DNA, 0.5 U Taq polymerase, 0.2 mM of each dNTP, 0.2 µM of VVMD5 forward and reverse primers, 2X PCR buffer, 2.5 mM MgCl<sub>2</sub> and 1X Q solution. The following thermal cycling protocol was applied for all loci: precycle 94 °C for 2 min; 35 cycles of denaturation for 1 min at 94 °C, 1 min of annealing at 50 °C and 1 min extension at 72 °C; postcycle of 30 min at 72 °C and then terminated at 4 °C. Amplified products were separated using ABI3130 Genetic Analyzer (Applied Biosystems, USA) with GeneScan-500 LIZ<sub>TM</sub> size standard. Sizing of the fragments was performed using GeneMapper 4.0 software (Applied Biosystems). Amplified profiles of reference cultivars were used for sizing the alleles of studied cultivars (THIS *et al.* 2004) which were afterwards coded according to GrapeGen06 methodology.

**Ampelographic description:** Ampelographic datasets were collected during 2009 and 2010 as is specified by the Organisation Internationale de la Vigne et du Vin (OIV 2001). Four OIV ampelographic descriptors were used to complement this study: OIV-225 (berry color), OIV-223 (berry shape), OIV-204 (bunch density) and OIV-204 (ripening - OIV 304), out of twenty-four ampelographic descriptors used in this project (003, 004, 016, 065, 068, 076, 079, 084, 085, 151, 202, 203, 204, 220, 223, 225, 230, 236, 241, 235, 504, 505 and 506). Ten readings per each descriptor were taken.

**Data analysis:** Data analysis was performed using GenAlEx 6.41 (PEAKALL and SMOUSE, 2006). The number of alleles per locus (Na), observed heterozygosity (Ho) and expected heterozygosity (He) were calculated for every sampled country. Polymorphism information content (PIC) was calculated using The Excel Microsatellite Toolkit 3.1.1. (PARK 2001). Specific alleles were detected using software CONVERT (GLAUBITZ 2004). Allelic richness was calculated with FSTAT (GOUDET 2001). Number of duplicates within and between sampling regions were tested with GenAlEx 6.41. Homonyms were detected by visual inspection. Probability of identity for unrelated gen-

Table 1  
Grapevine genetic diversity of South East European countries

Country	No of accessions	No of alleles	Allelic richness	Ho <sup>a</sup>	He <sup>b</sup>	No of unique genotypes	Specific alleles (locus, allele code)
Albania	10	5,67	5,54	0.821	0.731	9 (90%)	VVMD28, N+56
B&H Federation	12	7	6,3	0.787	0.739	8 (66.6%)	0
B&H Republika Srpska	13	6,33	5,8	0.825	0.714	8 (61.5%)	VVS2, N+32
Croatia <sup>c</sup>	15	6,89	5,98	0.729	0.759	15 (100%)	VVMD7,N+2; ZAG62, N+16; VVMD5, N ;
Macedonia <sup>c</sup>	14	6,56	5,86	0.777	0.768	13 (93%)	VVMD5, N+8; VVMD25, N+10; N+16;
Moldova <sup>c</sup>	16	6,67	5,64	0.724	0.745	12 (81.3%)	VVMD28 N+54
Montenegro	16	4,22	3,81	0.604	0.548	6 (37.5%)	0
Romania <sup>d</sup>	26	8,67	6,68	0.815	0.804	25 (96%)	VVMD7, N+26; VVMD27, N+16; VrZAG62, N+18; VrZAG79, N+24; VVMD5, N-2; N+2; VVMD25, N+8; VVMD28, N+2; VVMD32, N+9; N+13; N+19; N+39
Overall	122						

<sup>a</sup> Observed heterozygosity; <sup>b</sup> Expected heterozygosity; <sup>c</sup> Samples originate from official germplasm collections, address same as authors'. For Macedonia only two samples originate from official collection. <sup>d</sup> Samples originate from official germplasm collection: Research and Development Station for Viticulture and Oenology, Dragasani-Valcea Str. Regele, Ferdinand no. 64, Dragasani, County Valcea, 245700, Romania.

otypes, probability of identity for full sibs and frequency of null alleles were calculated using software IDENTITY4 (WAGNER AND SEFC 1999). Analysis of molecular variance (AMOVA) was used to analyze genetic variability within and between countries, using Arlequin 3.11 software (EXCOFFIER *et al.* 2005).

## Results and Discussion

Genetic diversity parameters (Tab. 1) were assessed for the analyzed genotypes across all SEE countries. Average number of alleles per sampling country varied between 4.22 (Montenegro) and 8.67 (Romania). Small average number of alleles as well as low values of other parameters for Montenegro plant material can be explained through the small number of different genotypes from this country analyzed in our study. Although initially 16 accessions were analyzed, only six different SSR profiles were detected in Montenegro.

The first step of analysis was limited only to accessions sampled within countries. Number of unique genotypes per country varied between 37.5 % (Montenegro) and 100 % (Croatia). Variation of allelic richness between countries was smaller, from 3.81 (Montenegro) to 6.68 (Romania). Observed heterozygosity was smaller than the expected one in two countries: Croatia and Moldova. For our dataset, analysis revealed that certain alleles can be found only among accessions from a single country, like Romania (twelve specific alleles), Croatia (three specific alleles) and Macedonia (three specific alleles). Information content of a given marker may vary between cultivars from different regions (LOPES *et al.* 1999) as confirmed by existence of specific alleles in our study.

All loci were very polymorphic and a total of 96 alleles were detected, ranging from 8 to 14 alleles per locus, with an average allele number of 10.67. Overall observed heterozygosity was 0.790 and slightly lower than expected (0.808) while gene diversity per locus varied between 0.600 (VVMD27) and 0.906 (VVMD28). The highest

number of alleles was detected at locus VVMD 28 (14). Loci VVMD 25 and VVMD27 had the lowest PIC values, 0.706 and 0.705 respectively. Cumulative probability of identity was  $9.42 \times 10^{-12}$  which indicates very low probability of two different varieties sharing the same genotype (Tab. 2). Microsatellite markers used in this study have been proven as very useful for cultivar identification. Six of them (VVS 2, VVMD 5, VVMD 7, VVMD 27, VrZAG 62 and VrZAG 79) are listed in the OIV primary descriptor list for identification of grapevine cultivars. In the large study of grape genetic diversity published by LAUCOU *et al.* (2011) seven of the used markers were the same as in our study. Analyzing 2323 accessions of *Vitis vinifera* subsp. *sativa* they detected 12 to 25 alleles per locus and loci VVMD 28 and VVMD 32 were the most polymorphic, as in our study. Comparing allelic richness of nine analyzed loci with a similar study of SEFC *et al.* (2000) who analyzed cultivars from geographically more distant European countries, we find genetic diversity of South East European countries to be relatively high.

In total 25 accessions showed to be duplicates (identical genotypes) within particular countries. Most of them ('Lipolist', 'Dupčara', 'Žilavka', 'Nadidžar', 'Vranac', 'Kratošija', 'Plavka') consisted of accessions with identical or very similar names having identical SSR profile. Among samples from Albania accessions 'Debine e zeze' and 'Koteke e zeze' had identical SSR genotype. Within samples from Macedonia accessions 'Ohridsko crno' and 'Stanushina' were found to be identical. Pairs of identical genotypes from Moldova were 'Brează' – 'Ciorcuță neagră' and 'Gordin' – 'Galbenă'. Accessions 'Surac' and 'Kadarun' from Republika Srpska as well as 'Krstač' and 'Bijela vinskā' from Montenegro were also found to be identical.

A subset of genotypes unique within each country of sampling consisted of 97 accessions and was subjected to further analysis. We searched for identical genotypes between countries and 11 pairs of crossborder synonyms were detected (Tab. 3). Most pairs of cultivars with identical genotype were detected among neighboring countries. Between two entities of Bosnia and Herzegovina two pairs

Table 2

Genetic diversity parameters of the nine microsatellite markers used in this study for 86 nonredundant accessions: number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content (PIC), probability of identity for unrelated genotypes ( $P_{ID}$  unrelated), probability of identity for full sibs ( $P_{ID}$  full sib) and frequency of null alleles (F (null))

Locus	Na	Ho	He	PIC	$P_{ID}$ unrelated	$P_{ID}$ full sib	F (null)
VVS 2	11	0.733	0.795	0.762	0.072	0.381	0.032
VVMD 5	12	0.812	0.873	0.854	0.032	0.326	0.030
VVMD 7	11	0.774	0.732	0.687	0.115	0.432	-0.027
VVMD 25	8	0.726	0.750	0.706	0.104	0.416	0.011
VVMD27	9	0.600	0.750	0.705	0.106	0.415	0.083
VVMD 28	14	0.906	0.899	0.884	0.021	0.309	-0.006
VVMD 32	12	0.842	0.839	0.813	0.048	0.349	-0.005
vrZAG62	9	0.849	0.790	0.760	0.072	0.385	-0.035
vrZAG79	10	0.869	0.844	0.820	0.045	0.345	-0.016
Mean:	10.67	0.790	0.808		9.42 <sup>e-12</sup>	1.33 <sup>e-4</sup>	

Table 3

Identical genotypes (accession names with respective country codes)

1	Debine e zeze (ALB05)	Koteke e zeze (ALB06)	
2	Zložder (BIH-FBIH08)	Bumba (CRO06)	
3	Ohridsko crno (MKD01)	Stanushina (MKD06)	
4	Kratošija (MKD08)	<b>Kratošija (MNE10)<sup>a</sup></b>	<b>Kratošija stara (MNE11)</b>
5	Vranec (MKD05)	<b>Rehuljavi vranac (MNE12)</b>	<b>Vranac (MNE01, MNE02, MNE03, MNE05, MNE06, MNE07, MNE08, MNE16)</b>
6	Razaklija (MNE15)	Crven valandovski drenok (MKD10)	
7	Begljarka bela (MKD14)	Coarnă albă (ROU01)	
8	Gordin (MDA01)	Galbena (MDA02)	Cârlogancă (Crâmpoșie veche) (ROU18)
9	Negru batut (MDA04)	Bătută neagră (ROU05)	
10	Turba plotnaia belaia (MDA06)	Cabasma (MDA11)	
11	Tamaioasa (MDA08)	Tămăioasă românească (ROU17)	
12	Breaza (MDA13)	Ciorcuta negra (MDA16)	Vulpe (ROU07)
13	Krstač (MNE09)	Bijela vinska (MNE14)	
14	Gordan (ROU12)	Gordin (ROU13)	
15	<b>Žilavka (BIH-RS12, BIH-RS13)</b>	<b>Žilavka (BIH-FBIH10, BIH-FBIH12)</b>	
16	<b>Plavka (BIH-RS04, BIH-RS05)</b>	Kadarun crveni (BIH-RS17)	
17	Surac (BIH-RS10)	<b>Kadarun (BIH-RS14, BIH-RS15)</b>	
18	Šljiva (BIH-FBIH06)	NN (BIH-RS18)	
19	<b>Lipolist (BIH-FBIH01, BIH-FBIH04)</b>		
20	<b>Dupčara (BIH-FBIH02, BIH-FBIH09)</b>		
21	<b>Nadidžar (BIH-FBIH03, BIH-FBIH07)</b>		

<sup>a</sup> Duplicates (same name and country of origin).

of synonyms were detected. Homonyms were detected based on visual inspection and few cases were observed. Accessions ROU12 and ROU13 from Romania named 'Gordin' and 'Gordan' shared identical genotype but accession MDA01 named 'Gordin' from 'Moldova' showed different SSR profile. The situation is even more complex with accessions named 'Šljiva'. Two accessions from the Federation of B&H named 'Šljiva' (BIH-FBIH05 and BIH-FBIH06) had different genotypes and did not match the genotype of accession named 'Šljiva' from Croatia (CRO08). However accession BIH-RS18 which was sampled as 'unknown' had the identical genotype as accession BIH-FBIH06. Accessions 'Rezaklija' from Republika Srpska and 'Razaklija' from Montenegro despite the very similar name did not have identical genotypes. It is important to note that cultivar names were often changed during history, because of various reasons, such as lack of knowl-

edge and proper maintenance of grapevine germplasm. In similar studies, the numbers of synonyms and homonyms detected were sometimes even above 30 %. CIPRIANI *et al.* (2010) found only 745 unique genotypes out of 1005 analyzed accessions, while LAUCOU *et al.* (2011) found 2323 different cultivars out of 3727 *sativa* accessions from the Vassal collection. The results of AMOVA showed no clear differentiation between countries (data not shown). We also analyzed differentiation among three more distinct geographic regions (Moldova-Romania; Albania-Macedonia and B&H-Croatia-Montenegro) and again no statistically significant difference among them was observed.

The remaining 86 unique SSR genotypes (Tab. 4) represent the nonredundant set of regional genotypes that was used for calculation of above mentioned genetic variation parameters shown in Tab. 2. Only a part of OIV descriptors' results (berry color, berry shape, bunch density and



Table 4  
List of the 86 South East European grapevine accessions representing nonredundant genotypes, OIV descriptors and SSR profiles

Country code and accession number	Accession name	Berry color OIV-225	Berry shape OIV-223	Bunch density OIV-204	Ripening OIV-304	VV52	VVMD5	VVMD7	VVMD25	VVMD27	VVMD28	VVMD32	ssrVrZAG62		ssrVrZAG79	
													Nr+14	Nr+20	Nr+14	Nr+20
ALB01	Kallmet	6	4	4	57	Nr+10	Nr+10	Nr+18	Nr+4	Nr+28	Nr+14	Nr+30	Nr+30	Nr+14	Nr+20	Nr+6
ALB02	Sheshi i zi	6	4	5	7	Nr+20	Nr+14	Nr+16	Nr+4	Nr+14	Nr+20	Nr+15	Nr+37	Nr+12	Nr+30	Nr+6
ALB03	Sheshi i bardhe	1	4	5	7	Nr+10	Nr+4	Nr+8	Nr+4	Nr+6	Nr+12	Nr+21	Nr+37	Nr+12	Nr+30	Nr+6
ALB04	Debine e bardhe	1	3	3	5	Nr+10	Nr+10	Nr+8	Nr+4	Nr+4	Nr+12	Nr+15	Nr+37	Nr+14	Nr+28	Nr+6
ALB05	Debine e zeze	6	4	3	5	Nr+10	Nr+10	Nr+8	Nr+4	Nr+6	Nr+12	Nr+15	Nr+37	Nr+14	Nr+28	Nr+6
ALB07	Serime e bardhe	1	3	3	5	Nr+20	Nr+10	Nr+8	Nr+20	Nr+28	Nr+30	Nr+27	Nr+37	Nr+14	Nr+14	Nr+20
ALB08	Pulez	1	4	7	7	Nr+10	Nr+4	Nr+8	Nr+4	Nr+6	Nr+30	Nr+15	Nr+17	Nr+14	Nr+28	Nr+14
ALB09	Serime e zeze	6	4	3	5	Nr+20	Nr+6	Nr+12	Nr+10	Nr+10	Nr+12	Nr+15	Nr+37	Nr+12	Nr+30	Nr+6
ALB10	Vloshi	3	4	5	7	Nr+10	Nr+10	Nr+8	Nr+4	Nr+6	Nr+12	Nr+15	Nr+37	Nr+14	Nr+28	Nr+6
BIH-FBH04	Lipolist	1	3	5	7	Nr+10	Nr+4	Nr+8	Nr+4	Nr+6	Nr+12	Nr+15	Nr+37	Nr+14	Nr+22	Nr+6
BIH-FBH05	Šljiva	6	3	7	7	Nr+10	Nr+14	Nr+12	Nr+6	Nr+20	Nr+12	Nr+17	Nr+21	Nr+14	Nr+20	Nr+18
BIH-FBH07	Nadždžar	2	4	5	7	Nr+18	Nr+10	Nr+8	Nr+4	Nr+6	Nr+12	Nr+21	Nr+23	Nr+22	Nr+28	Nr+14
BIH-FBH09	Dupčara	6	4	7	5	Nr+10	Nr+4	Nr+8	Nr+6	Nr+6	Nr+28	Nr+17	Nr+21	Nr+14	Nr+30	Nr+14
BIH-FBH11	Biatna	6	3	3	5	Nr+20	Nr+4	Nr+8	Nr+4	Nr+28	Nr+14	Nr+27	Nr+37	Nr+14	Nr+30	Nr+8
BIH-RS02	Dalmatinka	6	2	7	7	Nr+10	Nr+4	Nr+8	Nr+6	Nr+6	Nr+12	Nr+15	Nr+37	Nr+14	Nr+14	Nr+6
BIH-RS04	Plavka	6	2	7	3	Nr+12	Nr+10	Nr+8	Nr+4	Nr+4	Nr+32	Nr+17	Nr+29	Nr+14	Nr+26	Nr+6
BIH-RS07	Radovača	1	3	7	5	Nr+10	Nr+4	Nr+8	Nr+4	Nr+4	Nr+30	Nr+17	Nr+37	Nr+14	Nr+22	Nr+6
BIH-RS08	Rezaklija	2	7	7	5	Nr+16	Nr+6	Nr+8	Nr+4	Nr+4	Nr+8	Nr+27	Nr+29	Nr+14	Nr+20	Nr+8
BIH-RS13	Žilavka	1	2	5	5	Nr+10	Nr+10	Nr+8	Nr+8	Nr+16	Nr+30	Nr+32	Nr+38	Nr+14	Nr+14	Nr+12
BIH-RS15	Kadarun	6	2	7	7	Nr+12	Nr+4	Nr+8	Nr+4	Nr+20	Nr+14	Nr+21	Nr+37	Nr+14	Nr+20	Nr+14
BIH-RS16	Kadarun bijeli	1	2	7	5	Nr+20	Nr+18	Nr+8	Nr+14	Nr+20	Nr+18	Nr+15	Nr+29	Nr+14	Nr+30	Nr+14
BIH-RS18	Unknown	1	2	3	3	Nr+10	Nr+10	Nr+8	Nr+4	Nr+4	Nr+18	Nr+29	Nr+37	Nr+14	Nr+30	Nr+14
CRO01	Gustopapica ninska	6	6	5	7	Nr+20	Nr+4	Nr+8	Nr+4	Nr+4	Nr+30	Nr+42	Nr+45	Nr+14	Nr+22	Nr+14
CRO02	Muskat bijeli omiški	3	4	5	5	Nr+10	Nr+6	Nr+10	Nr+14	Nr+14	Nr+28	Nr+29	Nr+37	Nr+14	Nr+30	Nr+10
CRO03	Vlaški crljenak	5	2	9	5	Nr+10	Nr+10	Nr+2	Nr+4	Nr+4	Nr+6	Nr+5	Nr+21	Nr+22	Nr+22	Nr+10
CRO04	Bak	5	2	3	5	Nr+20	Nr+4	Nr+8	Nr+4	Nr+4	Nr+6	Nr+15	Nr+29	Nr+14	Nr+30	Nr+14
CRO05	Oskorušica	5	6	3	5	Nr+20	Nr+4	Nr+16	Nr+4	Nr+4	Nr+18	Nr+21	Nr+37	Nr+14	Nr+30	Nr+14
CRO06	Bumba bijela	1	2	7	7	Nr+10	Nr+14	Nr+18	Nr+4	Nr+4	Nr+8	Nr+17	Nr+37	Nr+14	Nr+22	Nr+14
CRO07	Svetljak	1	2	5	7	Nr+12	Nr+4	Nr+8	Nr+20	Nr+20	Nr+12	Nr+15	Nr+17	Nr+14	Nr+30	n.a.
CRO08	Šljiva	5	4	3	5	Nr+12	Nr+4	n.a.	Nr+20	Nr+4	Nr+12	Nr+15	Nr+17	Nr+14	Nr+30	n.a.
CRO09	Stradunska	1	2	3	5	Nr+10	Nr+12	Nr+18	Nr+4	Nr+4	Nr+6	Nr+15	Nr+29	Nr+12	Nr+14	Nr+14
CRO10	Palarniška hvarska	1	2	7	3	Nr+28	Nr+4	Nr+6	n.a.	n.a.	Nr+32	n.a.	n.a.	Nr+16	Nr+30	Nr+12
CRO11	Bašćan	5	2	5	7	Nr+22	Nr+4	Nr+16	Nr+6	Nr+6	Nr+32	Nr+62	Nr+62	Nr+16	Nr+28	Nr+12
CRO12	Kamenina	6	2	7	3	Nr+28	Nr+4	n.a.	Nr+28	Nr+4	Nr+38	Nr+62	Nr+62	Nr+14	Nr+30	n.a.
CRO13	Ošljevinna	1	2	7	5	Nr+10	Nr+14	Nr+18	Nr+4	Nr+20	Nr+30	Nr+42	Nr+45	Nr+14	Nr+30	Nr+22
CRO14	Vrbic	1	2	3	5	Nr+20	Nr+10	Nr+8	Nr+4	Nr+4	Nr+38	Nr+62	Nr+62	Nr+14	Nr+14	Nr+18
CRO15	Volarevo	1	2	3	5	Nr+10	Nr+4	Nr+16	Nr+4	Nr+4	Nr+18	Nr+38	Nr+38	Nr+14	Nr+28	Nr+22
MDA03	Copciac	6	1	3	7	Nr+10	Nr+20	Nr+8	Nr+4	Nr+4	Nr+6	Nr+12	Nr+32	Nr+14	Nr+28	Nr+12
MDA05	Sghihara	1	2	9	7	Nr+10	Nr+6	Nr+16	Nr+4	Nr+4	Nr+10	Nr+17	Nr+37	Nr+30	Nr+30	Nr+8
MDA09	Seina	6	2	5	7	Nr+12	Nr+4	Nr+8	Nr+4	Nr+28	Nr+6	Nr+15	Nr+17	Nr+14	Nr+30	n.a.
MDA10	Cianac verde	1	1	7	7	Nr+12	Nr+10	Nr+8	Nr+4	Nr+4	Nr+12	Nr+15	Nr+37	Nr+20	Nr+22	Nr+6
MDA11	Cabasma	1	2	5	5	Nr+12	Nr+4	Nr+8	Nr+4	Nr+20	Nr+10	Nr+12	Nr+37	Nr+20	Nr+22	Nr+6
MDA12	Negru de Akkertian	6	2	9	7	Nr+10	Nr+10	Nr+8	Nr+4	Nr+4	Nr+10	Nr+37	Nr+37	Nr+14	Nr+26	Nr+10
MDA14	Negru de Clușeni	6	2	3	7	Nr+20	Nr+6	Nr+14	Nr+20	Nr+20	Nr+6	Nr+21	Nr+37	Nr+30	Nr+30	Nr+10
MDA15	Țigvoasa	1	3	7	9	Nr+12	Nr+10	Nr+12	Nr+14	Nr+20	Nr+10	Nr+17	Nr+17	Nr+26	Nr+30	Nr+14
MDA17	Țoruțig roză	5	1	5	9	Nr+28	Nr+4	Nr+18	Nr+4	Nr+28	Nr+12	Nr+54	Nr+54	Nr+30	Nr+30	Nr+12
MKD02	Ohriško belo	1	3	7	7	Nr+20	Nr+12	Nr+16	Nr+16	Nr+20	Nr+32	Nr+17	Nr+17	Nr+20	Nr+30	n.a.
MKD03	Chaus	1	7	3	5	Nr+12	Nr+16	Nr+8	Nr+14	Nr+20	Nr+18	Nr+42	Nr+42	Nr+14	Nr+22	Nr+14
MKD04	Belo zimsko	1	4	5	9	Nr+12	Nr+12	Nr+12	Nr+14	Nr+14	Nr+10	Nr+15	Nr+17	Nr+12	Nr+26	Nr+14
MKD06	Stanushina	6	2	5	7	Nr+12	Nr+10	Nr+18	Nr+18	Nr+20	Nr+14	Nr+30	Nr+30	Nr+20	Nr+30	Nr+6
MKD07	Begljarka crna	6	2	7	5	Nr+10	Nr+12	Nr+8	Nr+4	Nr+4	Nr+6	Nr+15	Nr+37	Nr+20	Nr+20	Nr+6
MKD09	Bojanka	6	2	7	5	Nr+16	Nr+4	Nr+12	Nr+4	Nr+4	Nr+6	Nr+15	Nr+37	Nr+14	Nr+20	Nr+22
MKD11	Cm valandovski drenok	6	2	3	9	Nr+22	Nr+8	Nr+20	Nr+12	n.a.	n.a.	n.a.	n.a.	Nr+14	Nr+14	Nr+22
MKD13	Manastirsko belo	1	2	3	9	Nr+16	Nr+10	Nr+8	Nr+14	Nr+14	Nr+6	Nr+52	Nr+52	Nr+14	Nr+30	Nr+20
MKD15	Belovina	1	2	7	5	Nr+10	Nr+12	Nr+8	Nr+10	Nr+14	Nr+8	Nr+42	Nr+42	Nr+14	Nr+14	Nr+14
MNE04	Listkina	2	4	7	5	Nr+10	Nr+14	Nr+8	Nr+6	Nr+6	Nr+10	Nr+18	Nr+18	Nr+30	Nr+30	Nr+14
MNE11	Kratosija stara	6	4	9	5	Nr+10	Nr+4	Nr+16	Nr+4	Nr+4	Nr+6	Nr+17	Nr+21	Nr+14	Nr+22	Nr+14
MNE13	Čubrica	5	4	7	5	Nr+10	Nr+14	Nr+8	Nr+18	Nr+18	Nr+6	Nr+20	Nr+42	Nr+21	Nr+26	Nr+30
MNE14	Bijela vnska	4	9	7	5	Nr+10	Nr+16	Nr+18	Nr+14	Nr+4	Nr+6	Nr+20	Nr+42	Nr+14	Nr+26	Nr+22
MNE15	Razaklija	3	5	7	7	Nr+16	Nr+10	Nr+8	Nr+16	Nr+14	Nr+10	Nr+28	Nr+42	Nr+14	Nr+22	Nr+14
MNE16	Vranac	6	7	7	5	Nr+10	Nr+10	Nr+16	Nr+4	Nr+6	Nr+6	Nr+21	Nr+21	Nr+20	Nr+20	Nr+22

Tab. 4 continued

Country code and accession number	Accession name	Berry color OIV-225	Berry shape OIV-223	Bunch density OIV-204	Ripening OIV-304	VVS2	VVM5	VVM7	VVM25	VVM27	VVM28	VVM32	srVZAG62	srVZAG79
ROU01	Coamnă albă	1	3	9	5	N+10	N+16	N+8	N+4	N+4	N+6	N+18	N+14	N+14
ROU02	Coamnă neagră	3	3	7	5	N+14	N+14	N+16	N+8	N+4	N+10	N+28	N+14	N+14
ROU03	Țăia caprei albă	1	10	3	7	N+14	N+16	N+8	N+6	N+4	N+10	N+28	N+26	N+14
ROU04	Țăia caprei neagră	6	10	3	7	N+14	N+18	N+16	N+6	N+4	N+12	N+28	N+14	N+14
ROU05	Băluță neagră	6	2	7	5	N+10	N+10	N+8	N+4	N+10	N+10	N+17	N+14	N+14
ROU06	Negru Vărtos	6	2	9	5	N+10	N+22	N+18	N+4	N+6	N+10	N+30	N+30	N
ROU07	Vulpe	6	2	7	5	N+10	N+6	N+8	N+4	N+6	N+6	N+29	N+14	N+22
ROU08	Românie	6	2	5	7	N+12	N+4	N+6	N+20	N+19	N+19	N+37	N+14	N+22
ROU09	Slavița	1	2	3	5	N+10	N+10	N+16	N+6	N+4	N+6	N+23	N+30	N
ROU10	Om rău (Verdea)	1	7	3	7	N+20	N+12	N+18	N+4	N+6	N+6	N+23	N+30	N
ROU11	Fetească neagră	6	2	7	5	N+20	N+12	N+8	N+4	N+10	N+2	N+20	N+14	N+10
ROU13	Gordin	1	2	9	5	N+10	N+4	N+8	N+4	N+4	N+4	N+37	N+30	N
ROU14	Teșor	1	2	3	5	N+10	N+14	N+16	N+6	N+4	N+10	N+28	N+14	N+10
ROU15	Crămposie selecționată	1	2	7	5	N+12	N+4	N+18	N+6	N+6	N+6	N+21	N+30	N+14
ROU16	Braghină albă	1	2	3	5	N+20	N+14	N+16	N+4	N+6	N+6	N+26	N+28	N+22
ROU17	Tămăioasă românească	1	2	3	5	N+10	N+10	N+18	N+6	N+4	N+19	N+37	N+12	N+14
ROU18	Țărlogancă (Crămposie veche)	1	2	7	5	N+10	N+4	N+8	N+4	N+10	N+10	N+29	N+20	N+14
ROU19	Fetească albă	1	2	7	5	N+10	N+4	N+16	N+4	N+10	N+10	N+17	N+20	N
ROU20	Negru moale	6	2	7	5	N+10	N+28	N+8	N+4	N+14	N+16	N+17	N+14	N+22
ROU21	Călina	2	3	3	5	N+12	N+2	N+14	N+4	N+6	N+6	N+19	N+14	N+22
ROU22	Victoria	1	3	3	5	N+12	N+18	N+8	N+4	N+10	N+18	N+18	N+22	N+12
ROU23	Novac	6	2	5	5	N+10	N+18	N+18	N+4	N+10	N+14	N+28	N+30	N+14
ROU24	Alutus	6	2	5	5	N+10	N+20	N+6	N+4	N+14	N+14	N+21	N+26	N+20
ROU25	Vilorum	1	2	9	5	N+10	N+20	N+16	N+6	N+4	N+14	N+21	N+14	N+18
ROU26	Negru de Drăgășani	6	2	7	5	N+20	N+22	N+8	N+6	N+4	N+4	N+37	N+14	N

ALB – Albania, BIH-FBIH – Bosnia and Herzegovina Federation, BIH-RS – Bosnia and Herzegovina Republika Srpska, CRO – Croatia, MDA – Moldova, MKD – Macedonia, MNE – Montenegro, ROU – Romania.

ripening) is presented in this paper in order to provide a basic navigation through divergence of analyzed material, as well as their frequencies across region (Tab. 5). Berry color is almost equally split between green yellow (41.8 %) and blue black (41.0 %). Most common berry shape was "globose", bunch density was mostly dense to medium dense, while regarding ripening time medium to late varieties predominated.

It was interesting to take a closer look on expression levels of ampelographic data for accessions determined by SSRs as being synonyms or homonyms (data not shown). In case of 'Krstač' and 'Bijela vinska' from Montenegro that had identical SSR genotype, ampelographic results pointed on obvious difference in berry color (green yellow vs. grey), as well as in berry shape (narrow ellipsoid vs. horn shape). Similarly, synonym pairs 'Plavka' (BiH-RS05) and 'Kadarun crveni' (BiH-RS17) differed in berry color (blue black vs. rose), 'Kadarun' (BiH-RS14) had blue black and 'Surac' (BiH-RS10) green yellow berry color while 'Šljiva' (BiH-FBIH06) had blue black and accession 'NN10' (BiH-RS05) had green yellow berry color. These findings will require deeper ampelographic and more SSR analysis work in order to check if the observed diversity was a result of mutation or a human error. Three pairs of homonyms mentioned above had expectedly very similar phenotype what could explain appearance of different names.

An attempt to present diversity of studied accessions by most commonly used OIV descriptors is given in Tab. 5. Since the ampelographic description was performed in different countries by different evaluators (and without referent cultivars in some cases), frequencies of levels' of expression of different characteristics have to be taken by prudence. Still, they represent state of the art and provide a rough insight in overall diversity. Ampelographic description of same (synonym) cultivars performed as described above resulted in slight inconsistencies and pointed on necessity of additional characterization by molecular markers.

## Conclusions

The autochthonous grapevine cultivars from South East Europe analyzed in this study showed relatively high level of diversity in comparison with similar studies. No clear differentiation between countries was detected although several specific alleles were identified. Detected synonyms between neighbouring countries were mostly unknown before, but since South East European countries share common history, certain level of crossing between cultivars can be expected. However, few cases of accessions with identical SSR genotype but different phenotype might be berry color mutants. The obtained results should give a lead to ampelographers in the region to examine the level of phenotypic (di)similarity of detected synonyms and homonyms in more details with more independent samples. Prospection should be continued in the region of South East Europe to get better insight in relationships between cultivars and to preserve the existing germplasm for future generations. Accessions of unique and not previously published genotypes will be added to the European Vitis Database.

Table 5

Frequencies of some OIV characteristics for the 122 grapevine accessions presumed to be autochthonous across South East European countries

OIV characteristic	ALB	BIH-FBIH	BIH-RS	CRO	MDA	MKD	MNE	ROU	Total
	n = 10	n = 12	n = 13	n = 15	n = 16	n = 14	n = 16	n = 26	n = 122
Berry color - OIV 225									
1 - green yellow	40	41,7	46,2	46,7	50	42,9	6,3	53,8	41,8
2 - rose	0	16,7	15,4	0	0	0	6,3	3,8	4,9
3 - red	10	0	0	6,7	0	7,1	6,3	3,8	4,1
4 - grey	0	0	0	0	0	0	6,3	0	0,8
5 - dark red violet	0	0	0	33,3	12,5	0	12,5	0	7,4
6 - blue black	50	41,7	38,5	13,3	37,5	50	62,5	38,5	41
Berry shape - OIV 223									
1 - obloid	0	0	0	0	6,3	7,1	0	0	1,6
2 - globose	0	0	84,6	73,3	87,5	50	0	73,1	50,8
3 - broad ellipsoid	30	66,7	7,7	0	6,3	14,3	6,3	15,4	16,4
4 - narrow ellipsoid	70	33,3	0	13,3	0	7,1	37,5	0	16,4
5 - cylindric	0	0	0	0	0	0	6,3	0	0,8
6 - obtuse ovoid	0	0	7,7	13,3	0	21,4	0	0	1,6
7 - ovoid	0	0	0	0	0	0	43,8	3,8	9,8
8 - obovoid	0	0	0	0	0	0	0	0	0,8
9 - horn shaped	0	0	0	0	0	0	6,3	0	1,6
10 - finger shaped	0	0	0	0	0	0	0	0	1,6
Bunch density - OIV 204									
1 - very loose	0	0	0	6,7	0	0	0	0	0,8
3 - loose	50	8,3	7,7	40	12,5	0	0	34,6	19,7
5 - medium	40	50,3	23,1	20	37,5	64,3	12,5	11,5	29,5
7 - dense	10	41,7	69,2	26,7	37,5	35,7	68,8	34,6	41
9 - very dense	0	0	0	6,7	12,5	0	18,8	19,2	9
Ripening - OIV 304									
1 - very early	0	0	0	0	0	0	0	0	0
3 - early	0	0	7,7	13,3	0	0	0	3,8	3,3
5 - medium	50	25	76,9	60	12,5	50	93,8	80,8	59
7 - late	50	75	15,4	26,7	68,8	21,4	6,3	15,4	32
9 - very late	0	0	0	0	18,8	28,6	0	0	5,7

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### Authors' contribution

K. BELESKI was the project coordinator and dealt with analysis of overall ampelographic data; M. ŽULJ MIHALJEVIĆ performed molecular characterization, preliminary data analysis and wrote the manuscript; S. ŠIMON participated in molecular characterization and in writing the manuscript, I. PEJIĆ supervised final data analysis and made the concept of results and conclusions; all other co-authors participated at national level in ampelographic analysis and gathering plant material for DNA, as well as critical reading of the manuscript.

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