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MOLECULAR GENETIC ANALYSIS OF IDENTITY AND PARENTAGES IN WESTERN BALKAN GRAPEVINES (*VITIS VINIFERA* L.)

Abstract: Progress in genome sequencing and mapping studies have resulted in hundreds of polymorphic neutral markers, such as microsatellites, which have proven to be powerful tools for parental and kinship analysis, including grapevines. Microsatellites are one of the most exploited molecular markers in various research areas, including plant genotyping. The scope of our past analysis was determination of the identity and variability of autochthonous grapevines from the Western Balkan region. We analyzed presumed old and indigenous grapevine material collected in local vineyards, grapevine collections or individual farms from Serbia, Bosnia and Herzegovina, Macedonia, Montenegro and Slovenia.

A set of 196 accessions was genotyped at 22 microsatellite loci.

By intra-comparison of 196 profiles, 110 unique genotypes, 17 associations of duplicates, 21 of synonyms and 3 cases of homonyms were obtained. Further comparative analysis and origin assessment showed that 64 accessions with unique genotypes, prime names and putative synonyms can be assigned a Balkan origin. The majority of authentic genotypes were collected in Serbia and Slovenia, the two countries with

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established national germplasm collections, while the majority of accessions from Bosnia and Herzegovina are not found in any available database. The genotyping data of Balkan grapevines were integrated in the larger context of worldwide grapevine genotypes by comparison to datasets from large scale studies of genotyping [25], parentage analysis [23] and structuring [1] with the aim of revealing issues relating to the trueness-to-type, parentage and origin of some Balkan grapevines.

Key words: *microsatellites, Balkan grapevines, synonyms, identity, parentage*

INTRODUCTION

Standard ampelographic characterization enables identification of grapevine cultivars to some extent but many misnomers and incorrectly assigned identities still occur, often due to the presence of pathogens modifying the plant morphology, or simply misleading information in relation to local nomenclature. The introduction of DNA based markers has allowed more accurate identification and microsatellites have been widely used in grapevine analysis in recent decades, supporting and upgrading ampelographic/morphological analysis. Because of their ability to be highly polymorphic, their high abundance in the genome and their ongoing development and use in many different projects, microsatellites are a useful tool for individual fingerprinting, detection of genetic diversity, analyzing population structures and revealing identities and parentages.

Obtaining the accurate identity of cultivars and their broad characterization is important for enabling their efficient use and conservation. Identification of accessions is thus a crucial phase in the management of germplasm collections [25]. Different collections worldwide maintain various numbers of accessions and it is hard to estimate how many unique grapevine cultivars exist. The number of *V. vinifera* L. cultivars held in world collections is estimated to be 10,000 (Alleweldt and Dettweiler, op. cit.; [56]; Pelsy 2010), while some give numbers between 6,000 and 10,000 (Galet, op. cit.; [25]). Based on molecular analysis, the number of cultivars is actually much lower and is estimated to be about 5,000 unique genotypes [25, 40], many of which are closely related [56]. This difference between the two estimates reflects the high number of duplicates/synonyms, mostly resulting from cultivation in different climatic areas with different native languages, which has contributed to differences in designation and misleading overestimation [56]. High throughput analyses of some of the largest collections have recently been performed: 1,148 grapevine accessions from the national repository of Conegliano from Italy [9], 4,370 accessions from the INRA grape repository [25], 2,273 accessions of grape from the FEM collection San Michaele all'Adige in Italy [15]. The high redundancy identified in these studies and discrepancies

among data available about existing grapevine cultivars have led to an overestimation of the genetic diversity of grapevine. At the same time, some accessions are still conserved *in situ* or waiting to be collected in individual regions and countries. The greatest effort for safeguarding and enhancing germplasm collections has been performed in the scope of the European project GrapeGen06 and the establishment of the European Vitis Database [34].

In our previous studies we established a microsatellite profile for 196 accessions grown in the Balkans from old times, assessing their genetic diversity and clustering [51, 59] and investigated parentages [50]. The aim of the present study was to analyze the identity for defining the groups of synonyms, homonyms and misnomers in order to assign correct prime names to the cultivars out of 196 accessions Origin of the cultivars was analyzed based on correct identities and germplasm flow was evaluated. Reference grape cultivars were added in order to compare the Balkan cultivars with other genotypes existing in various grapevine microsatellite databases. Allele frequency-based calculations were used to assess the information content of specific microsatellite loci and to confirm the robustness of the analysis. Known pedigrees were validated. The main output of these analyses is the establishment of a set of unique genotypes representing possible Balkan germplasm, with assigned prime names and putative synonyms.

MATERIALS AND METHODS

PLANT MATERIAL

One hundred and ninety six (196) accessions of grapevines collected in vineyards in Bosnia and Herzegovina — BIH (75), Macedonia — MAK (18), Montenegro — MNE (19), Serbia — SRB (57) and Slovenia — SLO (27) were analyzed [51]. During the field inventory, one year-old dormant canes were collected and were forced to bud under laboratory conditions. The well-known cultivars Barbera, Cabernet Sauvignon, Chardonnay, Merlot, Pinot Noir, Sultanine and Touriga National (Centre of Plant Biotechnology and Breeding, Biotechnical Faculty, University of Ljubljana) were also analyzed and served as references for standardization and comparison with other *Vitis* databases.

MICROSATELLITE ANALYSIS

DNA was extracted as described by Štajner et al. [51]. In order to ensure reliable identification of cultivars, the most informative loci were selected based on previous studies: VVS2 [58], VVMD5 and VVMD7 [5], VVMD25, VVMD27, VVMD28 and VVMD32 [3], VrZAG62 and VrZAG79 [46],

VChr3a, VChr5a, VChr5b, VChr5c, VChr7b, VChr8a, VChr8b, VChr9a, VChr13a, VChr15a, VChr18a, VChr19a and VChr19b [9, 10]. PCR amplifications were performed according to the protocols described in Štajner et al. [51]. One μl of merged PCRs (of samples labeled with different dyes) was added to 0.5 μl of LIZ 600 size standard and 8.5 μl of Hi-Di formamide. Amplified alleles were separated by capillary electrophoresis on an ABI Prism 3730xl DNA analyzer and sized with GeneMapper software version 4.0 (Applied Biosystems).

DATA ANALYSIS

The allelic data of Balkan genotypes plus data of reference cultivars are deposited in the online database (http://vitis.genetika.si/) which enables standardization process and comparison and identification of genotyped samples. The data were statistically tested with Identity 1.0 software [46] to calculate the probability of identity ($PI = \Sigma pi^4 + \Sigma\Sigma (2pipj)^2$, where pi and pj are the frequencies of the ith and the jth alleles and $i \neq j$). This parameter calculates the probability of obtaining identical genotypes in pairs of cultivars for a given locus. The same program was used to generate a list of accessions with identical genotypes. The polymorphic information content (PIC) was calculated using Cervus 3.0 software [21], according to the formula ($1 - \Sigma pi^2 - \Sigma\Sigma 2pi^2pj^2$) (Bootstein et al. 1980).

Previously described parentages were also checked using Cervus 3.0 in order to assign parents to their offspring through a likelihood method [21]. A simulation run was performed based on allele frequencies, in order to generate a pair of parental genotypes and a series of random unrelated genotypes. In the parentage analysis, offspring are assigned the most likely parents with an 85% confidence level.

IDENTITY ANALYSIS

An intra-group comparison of allelic profiles for the 196 accessions was performed in order to identify redundant accessions (duplicates) and establish a set of unique genotypes. The unique genotypes were further compared to the cultivars genotyped worldwide (inter-comparative analysis) and available in various publications or on-line: national Vitis databases (Bulgarian Database — BD, Croatian Vitis Database — CVD, Greek Vitis Database — GVD, NCGR Davis Grape database in USA — UCD), WBCD — Western Balkans Vitis Database; the online *Vitis* International Variety Catalogue — VIVC and the European Vitis database. The prime name and origin of the cultivars were obtained by exploring the VIVC catalogue or based on available DNA reference profiles.

RESULTS AND DISCUSSION

MARKER ANALYSIS

The two parameters, high PIC (Polymorphic Information Content) and low PI (Probability of Identity) value, describing good differentiation ability for the loci, were calculated for 22 analyzed loci. Loci with a PI lower than 0.10 are considered to be the most informative (VVMD28, VChr8b, VVMD5, VrZAG79, VVMD32 and VChr3a) and the least informative are loci with a PI value > 0.24 (VChr13a, VChr15a and VChr7b). The least informative loci revealed an uneven distribution of frequencies of amplified alleles, which significantly decreased the discriminating power of the locus.

The probability of identical genotypes over all loci was $2.96\times10-^{20}$. Compared to some other studies, this parameter is very low, indicating a high capacity of the employed loci to differentiate among all accessions and low probability of obtaining false synonyms. Sefc et al. [45], for instance, reported a high discrimination power of nine SSR loci for identification of 164 grapevine cultivars from different European regions (including some Balkan countries), representing a PI value of $2.0\times10-^{9}$.

GEOGRAPHIC ASSIGNMENT OF ORIGIN

Comparative analysis of the allelic profiles of 196 accessions resulted in a redundant set of accessions (87) consisting of: I) duplicates, II) synonyms and III) homonyms and/or misnomers.

The results of identity analysis showed that various internationally recognized cultivars were introduced to the Balkans, where they were given local names. In addition to the comparative SSR analysis, we also checked the origin of accessions by surveying the VIVC online database (Tab. 1, Fig. 1 and 2).

Unique genotypes collected in Bosnia and Herzegovina were of diverse origin, with the majority of introduced cultivars being from neighboring Croatia and Serbia. Thirty-eight percent of accessions collected in BIH have a unique genotype and may therefore be of Bosnian (Yugoslavian) or still undefined origin (Tab. 1).

In Macedonia, the majority of table cultivars were introduced from the "East" (Greece, Moldova, Serbia and Turkey), while wine cultivars came from France and Croatia. Five cultivars, Belovina, Končanka, Manastirsko Belo, Ohridsko Belo and Stanušina, representing 45% of the analyzed accessions, can be considered to be of Macedonian origin (Tab. 1).

After removing duplicate genotypes from the analyzed set of cultivars, 5 accessions (56%) collected in Montenegro were of Montenegrin (Yugoslavian)

origin or not yet defined. Other cultivars had been introduced from Azerbaijan, Bulgaria, France and Romania (Tab. 1). Recently, Maraš et al. [31] reported on microsatellite analysis of seventy Montenegrin samples resulting in 14 unique genotypes; out of these five well-known cultivars were found, the others being minor, lesser-known cultivars.

In Serbia, 26 cultivars (54%) were described in VIVC as having a Serbian (Balkan) origin (Tab. 1). Some of them are newly bred cultivars with known parentage: interspecific (Bačka and Petra) and *V. vinifera* crosses (Godominka, Gročanka, Neoplanta, Probus, Radmilovački Muskat, Župljanka). The rest of the cultivars (17) could be considered to be old local or traditional cultivars authentic to Serbia. Many cultivars were introduced from the neighboring countries, Hungary and Bulgaria (Tab. 1). A few originated in the East, e. g., Armenia and Ukraine, while many of them came from the West (Austria, France, Germany and Italy). Bešlić et al. [2] generated reference genetic profiles for 12 traditional grapevine cultivars of Serbia and eight of them were assigned to the group of *Convar pontica suconvar balcanica*.

The Slovenian set of accessions is dominated by Italian cultivars, followed by Austrian, German and Hungarian cultivars. Slovenia borders all these countries except Germany, which explains the resultant introduction of foreign plant material to Slovenia, where cultivars were given local names. However, 61% of accessions collected in Slovenia proved to have a unique genotype, with Slovenian or undefined origin (Tab. 1).

Table 1. Prime names and origins of 110 non-redundant genotypes
Origin and prime name are mainly derived from the online VIVC database. * — Prime name obtained from DNA reference profiles publically available; ? — Unknown origin or identity of unique genotype; × — *V. vinifera* crossings; ×× — interspecific crossing.

Accession	Origin	Prime name given in VIVC (2015)	DNA reference	Country of collection	Berry color	Comment
Babić	Croatia	Babic	Maletic et al., 1999; Sefc et al., 2000; Zdunic et al., 2008	BA	Noir	VrZAG62 missmatch for one allele. Technical error; scoring error or possible parent offspring relationship.
Bačka ××	Serbia	Backa	*VIVC 21272*	RS	Rose	Identity also confimed by validation of parentagesis
Bagrina	?	?	Present paper; Stajner et al., 2014; WBVD	RS	Rose	Unique genotype Different from Braghinia Rose (VIVC 1644)

Accession	Origin	Prime name given in VIVC (2015)	DNA reference	Country of collection	Berry color	Comment
Bakator Beli	Romania	Ardeleanca	*VIVC 571*	RS	Blanc	
Begljerka Bela MAK = Bele Kozije sise SRB = Bijela Prosip BIH = Drenak Beli SRB	Moldova	Coarna alba	*VIVC2724*	MAK; SRB; BIH	Blanc	
Begljerka Crna	Macedonia	Begljerka Crna	WBVD; Zulj Mihaljevic et al., 2013	MAK	Noir	Confirmed by curator Beleski Klime
Bela Dinka	Serbia	Bela Dinka	Dzhambazova et al., 2012; WBVD	SRB	Blanc	Unique genotype
Bela Zgodnja SLO = Petrovka BIH	Hungary	Csaba Gyoengye	Santana et al., 2010; Ibanez et al., 2009; Sefc et al. 1999	BIH; SLO		VVMD28 one allele missmatch
Beli Medenac	?	?	Distinct from Honigler (VIVC 5417)	SRB	Blanc	Unique genotype
Belo Zimsko MAK = Zimsko Belo SRB	Greece	Opsimos Edessis	Štajner et al. 2009; GVD; VIVC	MAK; SRB	Blanc	
Belovina	Macedonia	Belovina	Stajner et al. 2014; WBVD	MAK	Noir	Unique genotype
Bena	BIH	Bena	WBVD	BIH	Blanc	
Blatina	BIH	Blatina	WBVD; Stajner et al. 2014	BIH; SRB	Noir	
Bojanka	France	Grand Noir	VIVC; Mena et al. 2014; Stajner et al. 2014	MAK	Noir	VVMD5 missmatching
Chaush = Čauš Crveni	Turkey	Chaouch Blanc	Riaz et al. 2014; Lacombe et al. 2013	MAK; SRB	Rose	Misnomer
Chaush Bel	Turkey	Chaouch rozovyi	VIVC	MAK	Blanc	Misnomer
Crn Valandovski Drenok	?	?	WBVD	MAK	Noir	Unique genotype
Crna Prosip	Yugoslavia	Lipolist	Present paper; Tomic et al. 2012; Stajner et al. 2012	BIH	Noir	Imperfect synonym
Crljenak BIH = Kratošija	Croatia	Primitivo	VIVC	BIH; MAK; MNE; SRB	Noir	Synonyms
Crven Valandovski Drenok MAK = Drenak BIH	Turkey	Parmak Cerven	VIVC	MAK; BIH	Rose	Mixed with Coarna Rosie a progeny from Parmak Cerven

Accession	Origin	Prime name given in VIVC (2015)	DNA reference	Country of collection	Berry color	Comment
Ćilibarka	Moldova	Tsitsa caprei	Stajner et al. 2014; Lacombe et al. 2012; Beslic et al. 2012	SRB		VVMD5
Dobrogostina	Yugoslavia	Lipolist	Curretn paper; Tomic et al. 2012; Stajner et al. 2015	BIH	Blanc	Imperfect synonym One parent to Zilavka (Stajner et al. 2015)
Dolga Pentlja	Slovenia	Dolga Petla	Stajner et al. 2014; WBVD; Stajner et al. 2008	SLO	Blanc	
Dolgi Grozdi	Slovenia	Pelena	Stajner et al. 2014; WBVD; Crespan et al. 2011	SLO	Blanc	Synonym
Drenak Crni	?	?	WBVD	SRB	Noir	
Drenjak BIH = Trnjak BIH = Trnjak krupni BIH	Yugoslavia	Ruderusa	Zdunic et al. 2013; WBVD	BIH	Blanc	Misnomer
Elezovka	?	?	not found	BIH		Unique genotype
Frankovka	Austria	Blaufraenki-sch	Mena et al. 2014; Sefc et al; WBVD	SRB	Noir	Synonym
Furmint **	France	Knipperle	Stajner et al. 2014; WBVD; Lacombe et al. 2012	SRB	Blanc	Misnomer
Gavran	Serbia	Grk Crni	Present paper; Stajner et al. 2014; WBVD	SRB	Noir	Synonym
Gnet Kras	Slovenia	Gnet Kras	Present paper; Stajner et al. 2014; WBVD	SLO	Rose	True name pending
Gnjet	Italy	Piccola Nera	Stajner et al. 2015; Stajner et al. 2014; WBVD	SLO	Noir	Synonym
Godominka ×	Serbia	Godominka	Current paper	SRB	Blanc	True name
Grk	Yugoslavia	Grk bijeli	Stajner et al. 2014; WBVD; Zdunic et al. 2013	BIH	Blanc	Synonym
Gročanka ×	Serbia	Grocanka	Present paper	SRB	Blanc	True name
Harslevelu	Hungary	Harslevelue	Stajner et al. 2014; WBVD; Lacombe et al. 2012; Jahnke et al. 2012	SRB	Blanc	Alternative spelling

Accession	Origin	Prime name given in VIVC (2015)	DNA reference	Country of collection	Berry color	Comment
Kadarka	?	?	Different from Kadarka Kek (VIVC 5898)	SRB		Unique genotype
Kadarka Bela	Hungary	Kadarka Feher	WBVD; Dzhambazova et al. 2009	SRB	Blanc	
Kadarun = Podbil I BIH	Italy	Francavida	VIVC	BIH		Kadarun Misnomer (as there is Kadarun in VIVC) Podbil synonym
Kambuša = Dalmatinka = Podbil IX BIH	France	Alicante Henri Bouschet	Lopes et al. 2006; VIVC	BIH	Noir	Kambusa — Synonym Dalmatinka Synonym Podbil — misnomer (given as a synonym to Francavidda) Podbil IX BIH vchr19a one allele missmatch
Kavčina	Slovenia	Kavcina Crna	VIVC	SRB	Noir	Different from Koelner blau;
Kokur Beli	Ukraine	Kokur Belyi	Lacombe et al. 2012	SRB	Blanc	
Kolana	Slovenia		not found	SLO		Unique genotype
Končanka	Macedonia	Koncanka	Štajner et al. 2009; WBVD	MAK		
Kreaca	Serbia	Kreaca	Beslic et al. 2012; WBVD	SRB	Blanc	True name
Krivača Bijela	Armenia	Krivalja Bijela	Zdunic et al. 2013; WBVD	BIH	Blanc	Synonym
Krivaja	?	?	Present paper	SRB	Blanc	Unique genotype
Krkošija	Serbia	Krkosija	not found	BIH; SRB	Blanc	True name
Krkošija Šupljica	Serbia		Present paper	SRB	Blanc	Unique genotype
Krstač	Montenegro	Krstac	WBVD	MNE	Blanc	True name
Kujundžuša	?	?	Present paper	BIH	Blanc	Unique genotype
Kunbarat	Serbia	Kunleany	?	SRB	Blanc	Misnomer
Lekant	France	Gamay Noir *	Bowers et al. 1999; Sefc et al. 2000; WBVD; VIVC	MNE	Noir	Synonym
Mala Blatina = Prošip	?	?	Present paper; GVD	BIH	Noir	Unique genotype
Malvazija	Croatia	Malvasia Istriana	Stajner et al. 2014; WBVD; Crespan et al. 2011; Stajner et al. 2008	BIH	Blanc	Synonym

Accession	Origin	Prime name given in VIVC (2015)	DNA reference	Country of collection	Berry color	Comment
Malvazija	France	Sauvignon Blanc	Present paper; Stajner et al. 2014; Mena et al. 2014	SLO	Blanc	Misnomer
Manastirsko Belo	Macedonia	Manastir-sko Belo	VIVC	MAK	Blanc	True name
Marburger	Austria	Neuburg-er *	Regner et al. 1996; Sefc et al. 2000	SLO	Blanc	Synonym
Menigovka I BIH	Italy	Italia *	Crespan et al. 1999; Sanchez-Escribano et al. 1999; Martin et al. 2003; La-combe et al. 2013	BIH		Synonym or misno-mer? Depending on what „ "real" Menigovka IX BIH is.
Menigovka IX BIH	Citronelle	France	VIVC DI Vech-hi Lacombe et al. 2013	BIH	Blanc	Synonym or misno-mer? Depending on what "real" Menig-ovka IX BIH is.
Muskat Otonel	France	Muscat Ot-tonel *	Sefc et al. 1998; Crespan et al. 2001; Moravcova et al. 2006	SRB	Blanc	Alternative spelling
Muskat Krokan	France	Muscat Fleur D'Oranger*	Schneider et al. 2008; Gianetto et al. 2010	SRB	Blanc	Synonym
Muškat Ruža	Italy or France	Sciaccarello OR Muscat Rouge De Madere	VIVC Lacombe et al. 2013	SRB	Noir	Synonym OR Sybling
Muštoš Feher	Balkan ?	Mustoa-sa De Mad-erat	Galbacs et al. 2009; WBV	SRB	Blanc	Synonym
Neoplanta ×	Serbia	Neoplanta	Present paper	SRB	Blanc	True name #
Neznana Bela	Serbia	Slankamen-ka Bela	Lacombe et al. 2012; Galbacs et al. 2009; Halasz et al. 2005	SLO		Unknown
NN				BIH		Same as SLJIVA
NPS1	Italy		Carimi et al. 2010	BIH		Unknown
Ohridsko Belo	Macedonia	Ohridsko Blanc	WBVD	MAK	Blanc	Synonym
Ohridsko Crno	Macedonia	Ohridsko crno	Present paper; WBVD; Stajner et al. 2009	MAK	Noir	True name
Petra ××	Serbia	Petra	Stajner et al. 2014; WBVD	SRB	Blanc	True name #

Accession	Origin	Prime name given in VIVC (2015)	DNA reference	Country of collection	Berry color	Comment
Plavac Mali	Croatia ***	Plavac Mali	Pejic et al. 2000; Zdunic et al. 2008; CVD; UCD	BIH	Noir	True name
Plavka	Croatia***	Plavina Crna	Maletic et al. 1999; Sefc et al. 2000; UCD; VIVC	BIH	Noir	Synonym
Plemenka Nova	Italy or France	Angelo Pirovano x Chasselas Rose	VIVC	SRB	Rose	Synonym
Plovdina Crna	?	?	Present paper	SRB	Noir	Unique genotype
Polšakica	Slovenia	Poljsakica *	Stajner et al. 2008; WBVD	SLO	Blanc	Alternative spelling
Portugizac	Austria	Portugieser Blau	Sefc et al. 1998; Crespan et al. 2011	SRB	Noir	Synonyms
Probus ×	Serbia	Probus	Present paper; WBVD	SRB	Noir	True name#
Prokupac	Serbia	Prokupac	WBVD; Lacombe et al. 2012; Beslic et al. 2012	BIH	Noir	True name
Prokupac **	Serbia	Prokupac x Terano	Present paper	SRB	Noir	Unique genotype
Prošip Bijela	Croatia	Vela Pergolla *	Sefc et al. 2001; Maletić et al. 1999; VIVC	BIH	Blanc	Synonym
Radmilovački Muskat ×	Serbia	Radmilovacki Muskat	Present paper; Stajner et al. 2014; Galbacs et al. 2009; Ibanez et al. 2009	SRB	Blanc	True name
Radovača	Bosnia and Herzegovina	Radovaca	Present paper; WBVD; Zulj Mihaljevic et al. 2013	BIH	Blanc	Unique genotype
Radovača	France	Afus Ali *	WBVD; Stajner et al. 2014; Lacombe et al. 2012;	MNE	Blanc	Misnomer
Rezaklija MNE = Rezaklija V BIH	Romania	Razachie Rosie	WBVD; Lacombe et al. 2012; Dzhambazova et al. 2012	BIH; MNE	Rouge	Synonym or Misnomer
Rebula Briska	Italy	Ribolla Gialla *	Cipriani et al. 2010; Crespan et al. 2011	SLO	Blanc	Synonym??? VVMD32 one allele diff

Accession	Origin	Prime name given in VIVC (2015)	DNA reference	Country of collection	Berry color	Comment
Rebula Portalis = Rebula Stara	Greece	Heunisch Weiss	Rusjan et al. 2010; Halasz et al. 2005; Stajner et al. 2011; WBVD; Galbacs et al. 2009	SLO	Blanc	Synonym???
Refosco	Italy	Refosco dal Peduncolo Rosso	Sefc et al. 2000; Cipriani et al. 2010	SLO	Rouge	Synonym
Refošk = Teranovka	Italy	Terrano	Sefc et al. 2000; Maletić et al. 1999; VIVC	SLO	Rouge	Synonym
Razaklija	?	?	Present paper	BIH	Rouge	Unique genotype
Ruzica VI MNE	Romania	Negru Virtos	Present paper; VIVC	MNE	?	Synonym Misnomer
Ruzica V MNE	Bulgaria	Pamid	Present paper; VIVC Dzhambazova et al. 2009	MNE	?	Synonym Misnomer
Ružica	Hungary	Koevedinka	Present paper; VIVC; WBVD	SRB		Synonym
Ružica Mirisava	?	?	Present paper	SRB	Rose	Unique genotype
Sipa	?	?	Present paper; WBVD	SLO	White	Unique genotype
Sjeraka	?	?	not found	MNE	White	Unique genotype
Slankamenka Crvena	Bulgaria	Pamid	Present paper; VIVC; Dzhambazova et al. 2009	SRB	Rose	Synonym
Smederevka	Bulgaria	Dimyat	Dzhambazova et al. 2009	BIH; SRB	Blanc	Synonym
Sremska Zelenika	Balkan	Sremska Zelenika	Present paper; Beslic et al. 2012; Lacombe et al. 2012; Halasz et al. 2005; Galbacs et al. 2009	SRB	Blanc	True name
Stanušina	Macedonia	Ohridsko Crno	Presetn paper; WBVD; Stajner et al. 2009	MAK	Noir	Imperfect Synonym; Syblings; Parent offspring
Star Refosk				SLO		
Stara Blatina	Bosnia and Herzegovina	Blatina	Present paper; Zulj Mihaljevic et al. 2013; Tomic et al. 2012	BIH	Noir	Synonym
Stara Žilavka	Yugoslavia	Lipolist	Present paper; Tomic et al. 2012; Stajner et al. 2015	BIH	Blanc	Imperfect synonym to Dobrogostina and Crna Prosip
Stari Rizling	?	?	Present paper	MNE		

Accession	Origin	Prime name given in VIVC (2015)	DNA reference	Country of collection	Berry color	Comment
Sura Lisičina	Austria	Blaufraenkisch	Mena et al. 2014; Sefc et al; WBVD	SRB	Noir	Synonym
Surac Plavi	?	?	Present paper	BIH	Noir	Unique genotype pending
Šabaš	Ukraine	Shabash	Stajner et al. 2014; WBVD; Lacombe et al. 2012	SRB	Blanc	Alternative spelling or Synonym ???
Šasla Crvena	France	Chasselas Rose	Present paper; WBVD; Moreno-Sanz et al. 2011	SRB	Rose	Synonym
Šipon	Hungary	Furmint	Present paper; WBVD; Lacombe et al. 2012; Jahnke et al. 2009; UCD	SLO	Blanc	Synonym ????
Šljiva	?	Sljiva Hercegovacka	Zulj Mihaljevic et al; WBVD;	BIH	Noir	Unique genotype
Tamjanika Crna	Balkan	Tamjanika Crna	VIVC	SRB	Noir	True name
Tamjanika Crvena	Balkan	Tamjanika Crvena	Present paper	SRB	Rouge	Unique genotype
Traminac Crveni	?	Gewuerztraminer	WBVD; Mena et al. 2014; Lacombe et al. 2012; Ibañez et al. 2003; UCD	SRB	Rouge	Synonym ?
Trbljan Beli	Italy	Mostosa	Stajner et al. 2015; Lacombe et al. 2012	SRB	Blanc	Synonym?
Vrban Crveni	?	?	Present paper; Stajner et al. 2014; WBVD	SRB	Rouge	Unique genotype. Different from Urban Rot in VIVC
Veltlinac Zeleni **	?	?	Present paper	SRB		Unique genotype
Vinje	?	?	Present paper; Stajner et al. 2014	SLO	Blanc	Unique genotype
Vitovska Grganja	Slovenia	Vitovska	Crespan et al. 2011; Stajner et al. 2011	SLO	Blanc	Misnomer????
Vranac	Montenegro	Vranac	Stajner et al. 2009; CVD; UCD	BIH; MNE; SRB	Noir	True name
Vranec	Montenegro	Vranac	Stajner et al. 2014; WBVD; present paper	MAK	Noir	Alternative spelling VVMD7 missmatch
Žametovka	Austria	Koelner Blau	VIVC	SLO	Noir	Synonym

Accession	Origin	Prime name given in VIVC (2015)	DNA reference	Country of collection	Berry color	Comment
Žilavka	BIH	Zilavka	Stajner et al. 2014; WBVD; Tomic et al. 2012a; Tomic et al. 2012b; Sefc et al. 2000; Maletic et al. 1999	BIH; MNE; MAK; SRB	Blanc	True name
Žižak	Azerbei-jdzan	Sysak	Stajner et al. 2014; WBVD	MNE	Blanc/ Noir ???	Synonym or True name???
Žlozder	Croatia	Medna	Stajner et al. 2014; WBVD	BIH	Blanc	Synonym
Žunić	Balkan	Zunic	Present paper; WBVD	SRB	Blanc	True name
Župljanka ×	Serbia	Zupljanka	Present paper	BIH; SRB	Blanc	True name#
Žuta Žilavka	BIH	Zilavka	Present paper	BIH	Blanc	Synonym

In general, the analyzed set of cultivars consisted of cultivars originating from ex-Yugoslavian countries, Romania, Turkey, Azerbaijan, Armenia, Moldova, Ukraine, Austria, France, Germany, Italy and Hungary. Fig. 1 shows the relative distributions for cultivars originating from Balkan countries and Fig. 2 for the introduced cultivars. It should be noted that the Balkan set consists of 64 cultivars out of 110 unique genotypes collected and originating in: Bosnia and Herzegovina (10), Bulgaria (3), Croatia (8), Greece (1), Macedonia (5), Montenegro (5), Serbia (29) and Slovenia (11) (Fig. 1).

On the basis of data on the site of collection (country) and assigned origin of cultivars, the highest percentage of authentic traditional cultivars are in Serbia, Slovenia and Bosnia and Herzegovina, while Macedonia and Montenegro had a fairly lower number of original cultivars. The advantage of Serbia and Slovenia is that they possess collections for the preservation of

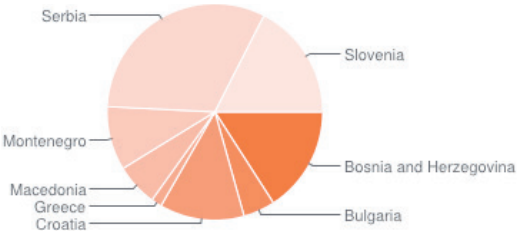

Figure 1. Relative distribution of Balkan unique genotype accessions in terms of collection and origin.

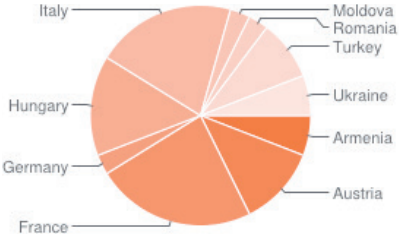

Figure 2. Distribution of introduced "eastern" or "western" cultivars in the Balkan region.

autochthonous grapevines and thus a higher number of well characterized and described cultivars. Many accessions from Bosnia and Herzegovina are not found in the VIVC catalogue; further investigation and evaluation of these uncharacterized cultivars is required. Historical data based on fossil remains that have been found in the Balkans may be an indication of independent domestication within these areas (Burić cit. op. [2]), as well as the presence of authentic grapevine materials. In terms of international cultivars found in the Balkan region (ex-Yugoslavia countries), there was a prevalence of French, Italian and Hungarian grapevines. During Ottoman rule over the Balkans, table cultivars were introduced from the Middle East, while, after the phylloxera recovery period, mainly cultivars from France were disseminated, as is also shown by the present results.

Molecular identification has been used in many different studies for discarding synonyms in order to obtain a unique set for diversity and origin analysis. The largest world collections, which contain more than several thousand accessions, have recently been characterized. Laucou et al. [25] analyzed 4,370 grapevine accessions on 20 microsatellite loci, obtaining 2,836 unique genotypes and 2,323 genotypes presented *sativa* cultivars used for further analysis of genetic structure [1]. Emanuelli et al. [15] obtained 1085 unique genotypes (out of 2273) and used them for further diversity assessment. Several other studies exanimating grapevine genotypes to smaller extent have been performed; Schneider et al. [43] reported that approximately half of the cultivars out of 45 examined were found to have a foreign counterpart mainly in other Mediterranean regions. In these studies, approximately half of the collections represented redundant accessions. In our case, redundant accessions represented by synonyms, homonyms and misnomers amounted to 44% (86 out of 196) of the collected set of genotypes.

CONCLUSIONS

The grapevine material collected in five Balkan countries represented old and traditional cultivars that are still preserved in local vineyards, nurseries or individual farms. Comparative analysis of the total set of 196 accessions showed that redundancy was 44% and that there are 110 unique genotypes. Analysis of the origin of the cultivars showed that 64 of the unique genotypes represent Balkan cultivars: Bosnia and Herzegovina (10), Bulgaria (3), Croatia (8), Greece (1), Macedonia (5), Montenegro (5), Serbia (16) and Slovenia (11). Eighteen out of the 64 unique Balkan genotypes represent authentic local material that is not found in the VIVC online catalogue of the world grapevine repository, which makes them priorities for further characterization (Drenjak = Trnjak = Trnjak Krupni, Elezovka, Gnet Kras, Gnjet,

Kolana, Manastirsko Belo, Menigovka, Muškat Ruža, Neznana Bela, Plovdina Crna, Radovača, Rezaklija, Ružica Mirisava, Sjeraka, Stara Žilavka, Stari Rizling, Surac Plavi and Vinje). An overview of the origin of the cultivars collected in Balkan countries demonstrates an already familiar trend: during the Ottoman Empire, new, mainly table cultivars were introduced from the Middle East, while after the phylloxera crisis, growth of French cultivars expanded. Parentage analysis was carried out to check known, newly bred hybrids and cultivars, while extended kinship analysis is of great importance for better understanding genetic structure in cultivated grapevines. These cultivars should be subject to continued evaluation in order to determine their standard representative traits, as the basis of their unique identity. Cultivars with unclear identity should be further investigated in order to determine their true varietal identities.

Overall, this study gave us the opportunity to describe the heterogeneity of Balkan grapevines, providing information on the introgression of foreign cultivars in the Balkans and determining synonyms, which has not previously been described to this extent.

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MOLEKULARNE GENETSKE ANALIZE IDENTIFIKACIJE I RODITELJSTVA KOD ZAPADNOBALKANSKIH SORTI VINOVE LOZE (*VITIS VINIFERA* L.)

Sažetak

Napredak u sekvencioniranju genoma i mapiranju rezultirao je stotinama polimorfnih neutralnih markera, kao što su mikrosateliti, koji su se pokazali kao moćni alati za roditeljsku i analizu srodnosti, uključujući i analize vinove loze. Mikrosateliti su jedan od eksploatisanih molekularnih markera u različitim područjima istraživanja, uključujući genotipizaciju biljke. Oblast naših prethodnih analiza je bila utvrđivanje identiteta i varijabilnosti autohtonih sorti vinove loze iz regiona zapadnog Balkana. Analizirali smo stari i potencijalno autohtoni materijal vinove loze prikupljen u lokalnim vinogradima, u kolekcijama vinove loze ili u pojedinačnim vinogradima iz Srbije, Bosne i Hercegovine, Makedonije, Crne Gore i Slovenije.

Komplet od 196 uzoraka genotipizovan je sa 22 mikrosatelitska lokusa.

Analiza identiteta 196 uzoraka otkrila je 110 jedinstvenih genotipova, 17 asocijacija duplikata, 21 sinonim i 3 slučaja homonima. Dalje komparativne analize i procjena porijekla pokazale su da se 64 uzorka sa jedinstvenim genotipovima, imenima i navodnim sinonimima mogu pripisati balkanskom porijeklu. Većina autentičnih genotipova sakupljana je u Srbiji i Sloveniji, dvije zemlje koje imaju formirane nacionalne kolekcije germplazme, dok se većina pristiglih iz Bosne i Hercegovine ne nalazi ni u jednoj dostupnoj bazi podataka. Podaci o genotipizaciji vinove loze na Balkanu integrisani su u širi kontekst svjetskih genotipova vinove loze u poređenju sa skupovima podataka iz obimnih studija genotipizacije analizom roditeljstva i strukturom s ciljem rješavanja pitanja vezanih za istinitost prema tipu, za roditeljstvo i porijeklo nekih sorti vinove loze sa Balkana.

Ključne riječi: mikrosateliti, balkanske sorte vinove loze, sinonimi, identitet, roditeljstvo