

Vitis 54 (Special Issue), 67–72 (2015)

Genetic clustering and parentage analysis of Western Balkan grapevines (*Vitis vinifera* L.)

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Summary

A total of 90 grapevine samples collected in five countries of the Western Balkan region were evaluated for trueness-to-type and kinship relations based on comparative analysis with 1,130 grapevine genotypes held at the INRA "Domaine de Vassal" French Grape Germplasm Repository, using 14 microsatellite markers. In the context of the comparative analysis, twenty-four synonyms/counterparts and the putative parents for twelve Balkan accessions were identified. We discovered five pairs of homonyms, subsequently confirming the identity or parentage of three of them. Some of the examined accessions were identified either on the basis of the genotypes found in the literature, or through parentage relationships revealed in this study. For the remaining fifty accessions we were unable to establish either their pedigree or to identify them on the basis of SSR profiles available elsewhere. Finally, the Balkan genotypes that were not well classified by synonymy or parentage analysis were further studied with a Principal Coordinate Analysis to reveal genetic clustering within larger datasets of genotypes. The graphical display of the individual and group distances showed that about forty accessions (85 %) are structured within a group of Balkan and Eastern Europe genotypes and only a minor proportion resulted in admixed population assignment.

Key words: microsatellites; identity; synonyms; principal coordinate analysis.

Introduction

In recent decades, progress in genome sequencing has enabled the reporting of hundreds of polymorphic neutral markers, such as microsatellites, which have proven to be powerful tools for parental and kinship analysis, including with grapevines. The largest grapevine parentage analysis to date was performed on a dataset of 2,344 unique genotypes (LAUCOU *et al.* 2011) (held in the INRA "Domaine de Vassal" French Grape Germplasm Repository). The full parentage of 828 accessions was identified using 20 microsatellite loci (LACOMBE *et al.* 2013). The second larg-

est study explored the parentage of 1,005 grapevines and the analysis of kinship uncovered 74 complete pedigrees, with both parents identified (CIPRIANI *et al.* 2010). However, other smaller parentage studies have been carried out in several countries (e.g. MENA *et al.* 2014). In our study, microsatellites were used to investigate the parentage and origin of Balkan grapevines collected in five Western Balkan countries - Slovenia (SVN), Serbia (SRB), Bosnia and Herzegovina (BIH), Macedonia (MKD) and Montenegro (MNE). To integrate and evaluate the SSR data of Balkan genotypes in a larger context, the dataset from a large scale parentage study (LACOMBE *et al.* 2013) was chosen for comparison. The dataset used in this work thus encompasses 1,130 genotypes from the INRA Domaine de Vassal grapevine repository (<http://www1.montpellier.inra.fr/vassal>; LACOMBE *et al.* 2013) and 90 genotypes from the Balkan region (<http://vitis.atcgilabs.com>). Panels of synonyms/counterparts and putative parentage-offspring trios were obtained, which are presented and related to some existing reports. The standardization and integration procedure of the two sets of data is described, together with issues related to the linking procedure. The discovered genetic clustering in comparison to the larger reference dataset, are discussed.

Material and Methods

Plant material and DNA analysis: The first step of this study was the identity and parentage analysis of grapevine genotypes from two recently published studies (LACOMBE *et al.* 2013, ŠTAJNER *et al.* 2013), using 7 microsatellite loci (VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VVS2) that were common to the two studies.

The obtained results suggested that 90 samples from the Balkan region (Tab. 1) are interesting for further analysis with an additional set of loci: VVMD21, VVMD24 (BOWERS *et al.* 1999a), VVIp31, VVIv67, VVIp60, VVIIn16, VVIb01 and VVIq52 (MERDINOGLU *et al.* 2005). Standardization of allele length and panel bins between the two datasets was carried out using common reference samples 'Cabernet-Sauvignon', 'Chardonnay', 'Merlot', 'Pinot Noir', 'Sultanine', and 'Touriga Nacional'. The procedure of plant material sampling and DNA isolation was as described in

Table 1
List of the 90 grapevine accessions from Western Balkans and their passport data

Accession Name	Country of sampling	Prime Name	Variety No. /IVC	Accession Name	Country of sampling	Prime Name	Variety No. /IVC
Babić	BIH	Babic	844	Muskat Ruža	SRB	Muscat Rouge de Madere	8249
Bačka	SRB	-	21272	Muštoš Feher	SRB	Mustoasa de Maderat	8311
Bela Dinka	SRB	-	16848	Neznana Bela	SVN	-	-
Bela Zgodnja	SVN	Csaba Gyoengye	9166	Ohridsko Belo	MKD	Ohridsko Blanc	21403
Beli Medenac	SRB	-	-	Plavac Mali	BIH	Plavac Mali	9549
Belo zimsko	SRB	Opsimos Edessis	8789	Plavka	BIH	Plavina Crna	9557
Belovina	MKD	-	42060	Plovkina Crna	SRB	-	-
Bena	BIH	-	1129	Poljšakica	SVN	-	17712
Blatina	BIH	Blatina	1454	Prokupac	BIH	Prokupac	9734
Crn Valand. Drenok	MKD	Parmak Cerven	8945	Prokupac	SRB	-	-
Čauš	MKD	Chaush Chernyi	7307	Prošip	BIH	-	24630
Čauš Bel	MKD	Chaouch Blanc	10196	Prošip Bijela	BIH	-	-
Dobrogostina	BIH	Trbjan	12619	Radovača	BIH	-	24598
Dolga Pentlja	SVN	Dolga Petla	17675	Radovača	MNE	Afus Ali	122
Dolgi Grozdi	SVN	Pelena, Vitovska	24459	Razaklija	BIH	-	-
Drenak	BIH	Grganja	-	Rebula	SVN	Ribolla Gialla	10054
Drenak Beli	SRB	-	16564	Rebula Portalis	SVN	-	-
Drenak Crni	SRB	Hora	5423	Refosco	SVN	Refosco dal Peduncolo Rosso	9987
Drenjak	BIH	Ruderusa	10327	Refošk	SVN	Refosk Terrano	12374
Elezovka	BIH	-	-	Rezaklija	BIH	-	24131
Furmint	SRB	Knipperle	6312	Ružica	SRB	Koevidinka	13727
Gavran	SRB	Gek Crni	5067	Ružica Mirisava	SRB	-	-
Gnet Kras	SVN	-	24599	Ružica V	MNE	Pamid	8899
Gnjet	SVN	Piccola Nera	9235	Ružica VI	MNE	-	-
Godominka	SRB	-	16003	Sipa	SVN	Osipka	17705
Grk	BIH	-	-	Slankamenka	SRB	Pamid	8899
Gročanka	SRB	-	5069	Crvena	BIH	Dimyat	5716
Harsleveli	SRB	Harslevelue	5314	Smederevka	SRB	-	15934
Kadarka	SRB	-	-	Sremska Zelenika	MKD	Stanusina Crna	11994
Kadarka Bela	SRB	Kadarka Feher	5899	Stara Žilavka	BIH	Trbjan	12619
Kadarun	BIH	Kadarun	5900	Stari Rizling	Montengro	-	-
Kavčina	SRB	Koelner Blau	6344	Surac Plavi	BIH	-	-
Kolana	SVN	-	-	Šipon	SVN	Furmint	4292
Končanka	MKD	-	22962	Šljiva	BIH	Šljiva	23206
Kratošija	MNE	Primitivo	9703	Tamjanika Crna	SRB	Hercegovacka Tamjanika Crna	8057
Kreaca	SRB	Kreaca	6501	Trbljan Beli	SRB	Trbjan	12619
Krivča Bijela	BIH	Krivalja Bijela	10050	Trnjak	BIH	-	-
Krivaja	SRB	-	-	Trnjak Krupni	BIH	Ruderusa	10327
Krkošija	BIH	-	16850	Urban Crveni	SRB	Urban	12781
Krkošija Šupljica	SRB	Kujundzusa Bela	6545	Vranac	BIH	Vranac	13179
Kujundžuša	BIH	Kujundzusa Bela	6545	Žametovka	SVN	Kavčina Crna	6047
Malvazija	BIH	-	-	Žilavka	BIH	Zilavka	13446
Manastirsko Belo	MKD	-	24597	Žižak	MNE	-	-
Marburger	SVN	Neuburger	8501	Žlozder	BIH	Medna	7584
Menigovka	BIH	Italia	5582	Žunić	SRB	-	15923

ŠTAJNER *et al.* (2013) as well as PCR amplifications and genotyping of SSR loci.

Data analysis: Amplified fragments were analysed and sized with GeneMapper software version 4.0 (Applied Biosystems, Carlsbad, California). All the phenograms were manually checked and the relative sizes of the amplified products were determined. Relative allele sizes were first standardized using six reference cultivars that were common to the two data sets; this procedure enabled us to jointly examine data from both sets of samples. To carry out this task, we wrote several custom R scripts

for improved management of input/output data, dealing specifically with the normalization of SSR data from two different sets and with identity analysis. The scripts, with instructions for use are freely available from <https://github.com/vp-uni-lj/ssr-link.git>. The first step consisted in the identification of matching genotypes, *i.e.* counterparts between the two datasets. The synonymous genotypes obtained by the R scripts were also manually inspected. Data were subsequently analysed with Cervus 3.0 software (MARSHALL *et al.* 1998; KALINOWSKI *et al.* 2007). Parentage analysis was performed in two steps; 1) we ran a simula-

tion of parentage analysis to estimate the resolving power of a series of microsatellite loci and 2) parentage analysis was performed to test candidate parents against offspring and, for each offspring tested, to assign the most-likely candidate parent with a pre-determined level of confidence, or left it unassigned. LOD (logarithm (base 10) of odds) scores were assigned to each possible parent and parent pair.

The genetic clustering of Balkan samples was obtained with a Principal Component Analysis (PCA) using the package *ade4* implemented in R (JOMBART 2008). The internal structure of the data and quantitative variables were calculated for predetermined groups of Vassal samples based on STRUCTURE clustering as reported by BACILIERI *et al.* (2013).

Results

Identity analysis: Based on the identity analysis, twenty-four Balkan genotypes (Tab. 2) found a counterpart in the Vassal dataset. Two pairs of near synonyms differing in one allele only were additionally identified: 'Beli Medenac' and 'Lisztesfeher' #2808 differed by 2 bp at locus VVIp60 and 'Gročanka' and 'Grocanka' #3637 by 4 bp at locus VVIb01. In addition, the counterparts were found among published SSR data for seven accessions: 'Kadarka Bela' = 'Kadarka Byala' (DZHAMBANOVA *et al.* 2009), 'Kreaca' = 'Banati Rizling' (GALBACS *et al.* 2009), 'Mustos Feher' = 'Mustos' (GALBACS *et al.* 2009), 'Tamjanika Crna' = 'Muskat Ruza Porecki' (MALETIĆ *et al.* 1999), 'Žametovka' = 'Schwarzgrobe' (SEFC *et al.* 2000), 'Zimsko Belo' = 'Karatsoba' (SEFC *et al.* 2000) = 'Karatsova Naousis' (Greek Vitis Database) = 'Opsimos Lefko' (Greek Vitis Database) and 'Prošip Bijela' = 'Volovnik' (ŠTAJNER *et al.* 2008) = 'Vela Pergolla' (MALETIĆ *et al.* 1999; SEFC *et al.* 2001).

Parentage analysis: Parentage analysis based on 14 microsatellite markers resulted in a panel of potentially interesting parent/offspring trios including Balkan genotypes that were shown to be in either parent or offspring relationship with genotypes from the Vassal collection (Tab. 3).

Principal Coordinate Analysis: Finally, the Balkan genotypes that were not uncovered by the meaning of synonymy were further analysed with PCA (Figure). The PCA analysis reflects the relationships among groups from Vassal collection based on the STRUCTURE clustering (A3 - Western and Central Europe genotypes; B3 - Far and Middle-East genotypes and C3 - Balkan and East Europe genotypes) and their distance to the Balkan genotypes referred as D3 group. The PCA grouping shows that D3 Balkan samples are positioned close to the C3 samples that represent Balkan and East Europe genotypes in the study of BACILIERI *et al.* (2013).

Discussion

Most of the synonyms identified in this study (Tab. 2) were not reported in the relevant papers published recently

Table 2

List of identical grapevine genotypes based on matching allele sizes for analysed loci

Balkan sample	INRA sample (LACOMBE <i>et al.</i> 2013)
Bela Zgodnja (SVN)	Perle de Csaba (#1069) (HUN)
Čauš Bel (MKD)	Chaouch rose (#1674) (TUR)
Drenak (BIH)	Rosa menna di vacca (#1662) (ROU)
Drenak Beli (SRB)	Coarna alba (#749) (ROU)
Drenak Crni (SRB)	Darkaia noir (#728) (MAR)
Elezovka (BIH)	Chaouch blanc (#1673) (TUR)
Furmint (SRB)	Knipperle (#283) (FRA)
Gnjjet (SVN)	Piccola nera (#2387) (ITA)
Harslevelu (SRB)	Harslevelu (#1609) (HUN)
Kratosija (MNE, MKD)	Primitivo = Zinfandel (#1277) (ITA)
Marburger (SVN)	Neuburger (#2172) (AUT)
Menigovka (BIH)	Italia = Pirovano 65 (#926) (ITA)
Muskat Ruza (SRB)	Muscat rouge de Madère = Moscato violetto (#576) (ITA)
Plavka (BIH)	Plavina crna (#1843) (HRV)
Prokupac (BIH)	Prokupac (#1630) (SCG)
Radovaca (MNE)	Dattier de Beyrouth = Afuz Ali (#634) (TUR)
Refosk (SVN)	Terrano (#1293) (ITA)
Rezaklija' (BIH)	Razachie rosie (#1887) (ROU)
Ruzica (SRB)	Kovidinka (#1578) (YUG)
Šipon (SVN)	Furmint (#25) (HUN)
Smederevka (SRB)	Dimiat (#1666) (BGR)
Srem Zelenika (SRB)	Szeremi zöld (#1623) (HUN)
Trbljan Beli (SRB)	Mostosa = Empibotte bianco (#2054) (ITA)
Žilavka (BIH)	Zilavka (#1637) (BIH)

(BEŠLIĆ *et al.* 2012, ZDUNIĆ *et al.* 2013, MARAŠ *et al.* 2014, SCHNEIDER *et al.* 2014). Among twenty-four associations found within two studies, the highest part of the Vassal counterpart are of Balkans origin (67 %) and the rest, except two, are of Italian origin for which BACILIERI *et al.* (2013) reported high level of admixture as regards to genetic composition of geographic origin.

The parentage analysis resulted in identification of parentages for twelve authentic Balkan genotypes having a parent either from our set of samples or from the Vassal collection and to our knowledge they have not yet been described in the literature. All varieties from Vassal have been assigned a geographic origin presented in Tab. 3, where we can see that even when one parent is identified in Vassal, still, most of the time it is of Balkans origin. The only notable exceptions are two parental accessions, one of which is 'Terrano' that is considered of Italian origin, but is widely grown also in Slovenia and Croatia and the second is 'Malvasia del Chianti' being part of a Malvasia group derived from Greece. Another two parental accessions of non-Balkan origin are 'Gouais blanc' = 'Heunisch weiss', one of the oldest varieties in the world, used in several crosses and 'Rosenmuskat' a Muscat used to introduce the muscat flavour.

Three out of twelve offspring described in parentage analysis are table grapevines ('Gročanka', 'Krivaja', and 'Šljiva') and their candidate parents are also table grapevines. The same findings were reported by LACOMBE *et al.* (2013), who stated that comparison of the fruit use of parents for a given progeny showed a majority of crosses us-

Table 3

List of full parentages detected for Balkan grapevine accessions. The symbol # refers to the accession number in the Vassal collection (http://bioweb.ensam.inra.fr/collections_vigne/)

Offspring	First candidate	Second candidate	Trio loci compared	Trio loci mismatching	Trio LOD score
Dobrogostina (BIH) = Stara Žilavka (BIH)	Krkošija (BIH)	Rosenmuskat (#3550) (DEU)	14	0	20
Godominka (SRB)	Muscat Ottonel (#280) (FRA)	Smederevka (SRB) = Dimiat (#1666) (BGR)	14	1	23
Gročanka (SRB)	Bela Zgodnja (SVN) = Perle de Csaba (#1069) (HUN)	Radovaca (MNE) = Dattier de Beyrouth = Afuz Ali (#634) (TUR)	15	0	30
Krivaja (SRB)	Drenak (BIH) = Rosa menna di vacca (#1662) (ROU)	Rezaklija (BIH) = Razachie rosie (#1887) (ROU)	14	0	23
Manastirsko Belo (MKD)	Drenak (BIH) = Rosa menna di vacca (#1662) (ROU)	Heptakilo (#743) (GRC)	14	0	31
Polšakica (SVN)	Malvasia del Chianti (#1352) (ITA)	Prošip Bijela (BIH)	14	1	27
Prokupac (SRB)	Prokupac (BIH) = Prokupac (#1630) (SRB)	Refošk (SVN) = 'Terrano' (#1293) (ITA)	14	0	32
Šljiva (BIH)	Drenak Beli (SRB) = Coarna alba (#749) (ROU)	Heptakilo (#743) (GRC)	14	0	27
Žilavka (BIH)	Albaimputotato (#44) (ROU)	Dobrogostina (BIH)	14	0	21
Bagrina (SRB)	Beli Medenac (SRB)	Braghina=Dinkavoros (#1670) (HUN)	13	0	17
Kreaca (SRB)	Drenak Beli (SRB) = Coarna alba (#749) (ROU)	Plavac Mali (#3144) (HRV)	14	1	14
Prošip (BIH)	Drenak Beli (SRB) = Coarna alba (#749) (ROU)	Gouaisblanc = Heunisch weiss (#211) (FRA)	14	0	13

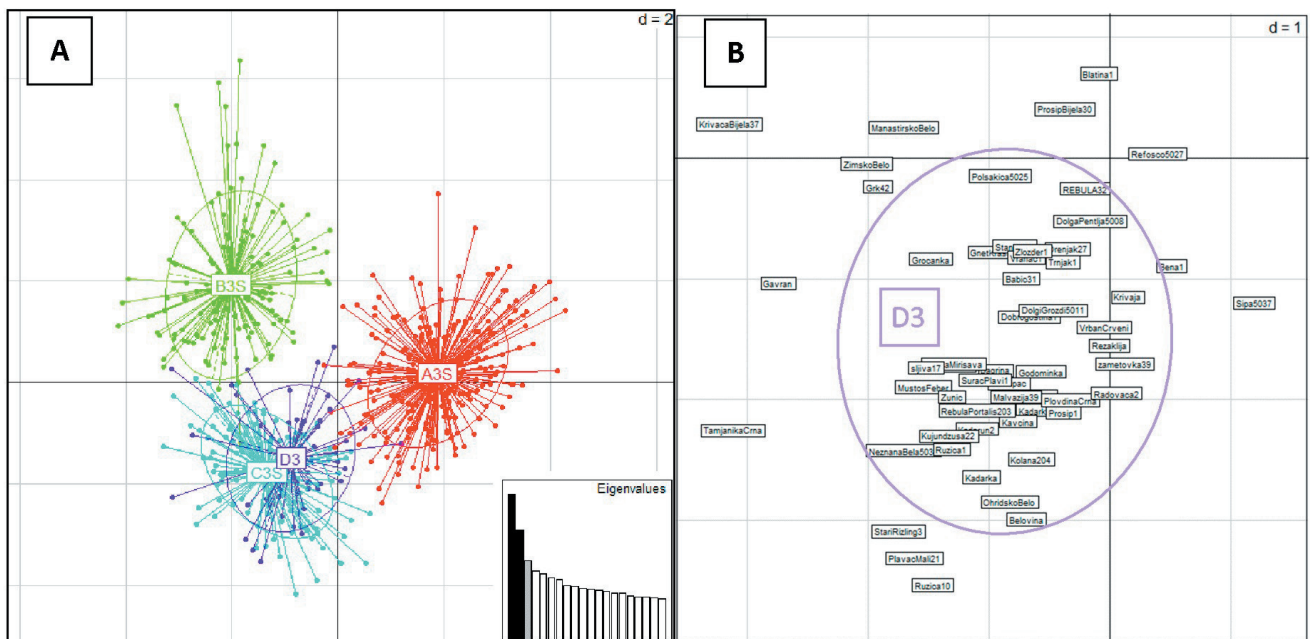


Figure: Principal component analysis on joint SSR data **A**) for non-identified Balkan samples (D3 group) and samples from Vassal collection subdivided in 3 structure groups as in *BACILIERI et al.* (2013): A3S, Western and Central Europe genotypes, B3S, Far- and Middle-East genotypes, and C3S, Balkan and East Europe genotypes) and **B**) for samples of D3 group graphically displaying their individual distances shown on the same axes as in Figure A; it presents the enlarged picture of Figure A, including names of accessions.

ing parents from the same geographical area and with the same grape use. The main Balkan genitors identified in our analysis are 'Heptakilo' (#743), a traditional table cultivar from Greece and 'Rosa menna di vacca' (#1662), a traditional table cultivar from Romania. These findings are in agreement with the historical view; grapevines arrived in the Balkan Peninsula from Central Asia via the Greek is-

lands and the Romans continued to spread the cultivation of grapevine on the Adriatic islands and on the mainland. During the period of the Ottoman Empire, the production of table grapes increased and was more widespread over the region (SAVIĆ 2003).

Several interesting hypotheses have arisen concerning some putative trios; introduced cultivars have often been

given local names and have eventually been considered native old cultivars. For example 'Žilavka' was considered to be a very old Bosnia and Herzegovina authentic cultivar. The parentage analysis by LACOMBE *et al.* (2013) showed one of the progenitors of 'Žilavka' to be 'Furmint' (#25) or 'Alba imputotato' (#44) or 'Goher' (#2244). MALETIĆ *et al.* (1999) reported that DNA profiling has suggested a genetic relationship between 'Žilavka' and 'Teran Bijeli' ('Prosecco') grown in Istria. Our results suggest that 'Žilavka' might be the offspring of an old female Romanian genitor 'Alba imputotato' (#44) and another BIH accession 'Dobrogostina' = 'Stara Žilavka' (Tab. 3). There are no formal records on breeding and selection of 'Žilavka', despite it being a very famous grapevine, particularly in BIH, and having been grown for more than 600 years in the region of Herzegovina. The Bosnian King Tvrtko was mentioned as drinking 'Žilavka' wine in the 14th century (VUKSANOVIĆ and KOVAČINA 1984). Wine made from 'Žilavka' is economically important and very popular in BIH and also in some adjacent regions of Croatia, Macedonia, Montenegro and Serbia, where it is also grown (TOMIĆ *et al.* 2012).

In the field of grapevine studies, there is still an ongoing debate about the origin of the synonymic genotypes: Californian 'Zinfandel', Croatian 'Crljenak Kaštelanski', Montenegrin 'Kratošija' and Italian 'Primitivo' (MALETIĆ *et al.* 2004; CALÒ *et al.* 2008; LACOMBE *et al.* 2007; CIPRIANI *et al.* 2010). Some data suggest a possible first-degree relationship between the cultivars 'Kratošija' and 'Vranac' (MALETIĆ *et al.* 2004, CALÒ *et al.* 2008) and a parent-offspring relationship of 'Kratošija' with 'Plavina' and 'Vranac' has been proposed (MALETIĆ *et al.* 2004; CALÒ *et al.* 2008). Others have suggested that 'Plavina' (= 'Plavina crna') could be an offspring of 'Kratošija' and 'Verdeca', a white cultivar grown in southern Italy (LACOMBE *et al.* 2007, CIPRIANI *et al.* 2010, LACOMBE *et al.* 2013). In our analysis, two accessions of 'Kratošija', originating from Montenegro and Macedonia, resulted in identical allelic profiles and were therefore confirmed as synonyms and, on the basis of comparison to genotypes of the Vassal collection, they were also confirmed as synonyms of the cultivar 'Primitivo' = 'Zinfandel' (#1277). In our parentage analysis, 'Vranac' and 'Plavina crna' (#1843) were one of the most likely candidate parent pairs for 'Kratošija' (results not shown) but, in a comparison to the trio proposed by LACOMBE *et al.* (2013), by which 'Plavina crna' is an offspring of 'Kratošija' (= 'Primitivo' = 'Zinfandel') and 'Verdeca' (= 'Lagorthi'), the latter resulted in a higher LOD score value than the former, with assigned LOD scores of 22.5 and 15.2, respectively. 'Kratošija' is an old Montenegrin grape accession that, according to the literature, existed in these regions before 'Vranac', although 'Vranac' today predominates in Montenegro and is often cultivated together with 'Kratošija' (ULIČEVIĆ 1966, PEJOVIĆ 1988, MARAŠ *et al.* 2003, 2004, 2014). MARAŠ *et al.* (2014) evidenced that 'Kratošija' with all its biotypes has been grown in Montenegro for centuries and suggested that this region be considered a likely region for the origin and spreading centre of 'Kratošija'. According to STRUCTURE clustering reported by BACILIERI *et al.* (2013) cultivars 'Kratošija' and 'Vranac' were considered as admixed, with 61 % and 72 % of its genome belonged

to S-3.3 cluster (Balkan and Eastern Europe group), respectively. During the first inventory of Balkan samples, we found that 'Prokupac' obtained from the Sremski Karlovci (Serbia) collection vineyard (where this cultivar is also bred) resulted in a distinct allelic profile as 'Prokupac' from Davis collection, (USA), but they shared 73 % of alleles analysed at 22 loci (ŠTAJNER *et al.* 2013). The present analysis similarly confirmed that 'Prokupac' from Serbia is different from 'Prokupac' (#1630) from the Vassal collection. In contrast, another 'Prokupac' from BIH resulted in a genotype identical to Prokupac from Davis collection and Prokupac from Vassal collection. The parentage analysis revealed that 'Prokupac' from the Sremski Karlovci collection vineyard might be a progeny of "true-to-type" 'Prokupac' and 'Terrano', showing high LOD scores (31.6) and no allele mismatch. A possible explanation for this misnomer is that progenies of a particular parent were sometimes treated the same as the parent cultivar due to misleading documentation of events or possible mislabelling.

The PCA analysis shows that Balkan samples (D3, Figure A) overlapped with the genotypes from Balkan and Eastern Europe (C3S group). Hence, the genotypes graphically displayed inside the D3 ellipse might be characterized as typically autochthonous Balkan accessions (Figure B). On the other hand, the accessions that are out of ellipse and are classified between two different groups, e.g. 'Blatina' and 'Prosip Bijela' probably are recent crosses between one table and one Western genotype, with one parent in the A3 and the other in the B3 group. Few of the analysed Balkan accessions are shown to be near to either A3 group ('Bena', 'Sipa', 'Refosco') or B3 group ('Manastirsko Belo', 'Krivača Bijela', Figure B), which reflects their admixed origin and corresponds to various breeding activities in the Western Balkan region. However, these non-Balkan accessions represent a minor proportion of the dataset, while about forty (85 %) analysed cultivars are considered true autochthonous Balkan germplasm.

Conclusions

Parentage analysis and PCA showed to be very valuable tools for identification of relationships. Many of the parentages revealed in this study were previously not known and are of considerable historical interest. The PCA showed that 44 accessions (85 %) are structured within a group of Balkan and Eastern genotypes and only a minor proportion resulted in admixed population assignment (Figure B). However, the identity of approximately forty Balkan accessions remains only partially explained so far. Some of these are presumably old traditional grapevines, with a putative Balkan origin, and are therefore worth exploring for their origin and identity.

Acknowledgements

The research was funded by the European Commission's Joint Research Project of the Joint Call of the SEE-ERA.NET

PLUS, grant no. ERA 155/01 and by the Slovenian Research Agency, grant no. P4-0077. The study was conceived within the framework of FA COST Action FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding".

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