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Ampelographic and molecular characterisation of grapevine varieties in the gene bank of the experimental vineyard 'Radmilovac' – Serbia

Kristina Milišić[1*], Branislava Sivčev[2], Nataša Štajner[3], Jernej Jakše[3], Saša Matijašević[2], Dragan Nikolić[2], Tatjana Popović[4] and Zorica Ranković-Vasić[2]

[1]Directorate for Agricultural Land, Gračanička 8, 11000 Belgrade, Serbia
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Serbia
[3]University of Ljubljana, Biotechnical Faculty, Jamnikarjeva cesta 101, Ljubljana, Slovenia
[4]University of Montenegro Faculty of Biotechnology, Mihaila Lalića 1, 81000 Podgorica, Montenegro

**corresponding author*: kiki.valjevo9@gmail.com

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Characterisations of thirty grapevine varieties (*Vitis vinifera* L.) from the experimental vineyard 'Radmilovac' were conducted using a large number of OIV descriptors and eight highly polymorphic microsatellite loci. The ampelographic description contained 45 features. Molecular characterisation of selected microsatellite loci was performed using capillary electrophoresis fragment analysis. Dendrograms based on ampelographic and genetic data resulted in three groups of varieties. Qualitative ampelographic characteristics tended to manifest significant differences. The most common deviation among varieties within the group was in the characteristic OIV 051 (colouration of the upper side of a young leaf). Genetic characterisation of SSR markers through analyses of a large number of varieties contributes to better organisation of grapevine collections and simpler identification of varieties, as well as data exchange. When identifying the varieties, the results of the DNA analysis should be combined with the ampelographic descriptors, in order to select grapevine varieties with desirable viticultural and oenological traits. Integration of the obtained genetic data with the ampelographic data is of utmost importance for accurate identification of the varieties and offers a significant means for the preservation and use of the varieties.

KEYWORDS

Vitis vinifera, variety, identification, OIV descriptors, SSR.

INTRODUCTION

Grapevine is an important horticultural species that is grown all around the world in temperate and tropical climates (Nikolić *et al*., 2015, 2018b). Grapes are consumed in a number of ways, including fresh or dried, fermented into wine and distillates, and pressed for fresh juice and jam. The most represented varieties in Serbia are Cabernet-Sauvignon, Merlot, Chardonnay and Sauvignon blanc covering 61 % of the cultivated area, while the indigenous variety Prokupac accounts for only 2 % of vineyards (Jakšić *et al.*, 2015).

Worldwide, a large number of varieties are grown for different purposes: an estimated 9,500 varieties for wine, nearly 4,500 varieties for fresh consumption, more than 1,200 varieties for both wine and fresh consumption, and about 110 varieties for drying (Töpfer *et al.*, 2011). Despite the large number of varieties in many breeding programmes, new cultivars with higher yields and fruit quality are constantly being created (Nikolić *et al*., 2015). Hybridisation is the most suitable method for creating new varieties of grapevine, as well as for researching the mode of inheritance for certain traits (Milutinović *et al*., 2000; Nikolić *et al*., 2018a). Grapevine breeding is a long-term process (Nikolić *et al.*, 2018b), and new crossings should be evaluated at least twenty-five years before being released to the public (Regner *et al.*, 2004). In 1984, the *Vitis* International Variety Catalogue (*V*IVC) was founded (Alleweldt, 1988). According to Maul *et al.* (2014), *V*IVC is an encyclopaedic database containing nearly 23,000 primary names and 42,000 synonyms of various species and varieties/cultivars of vines. Additionally, the intergovernmental International Organisation of Vine and Wine (OIV) has published a guide for identifying varieties (2009). Through these publications, a degree of coordination has been achieved in the descriptors adopted by the International Plant Genetic Resources Institute (IPGRI), the Unión Internacional para la Protección de las Obtenciones Vegetales (UPOV) and the OIV. The former Yugoslav Plant Genetic Resources Bank was created between 1989 and 1991. Through analysis of genetic material for the genus *Vitis*, a rich vine germplasm was established from a total of 13 collections in localities situated in temperate-continental and Mediterranean climates (Cindrić *et al.*, 1997). This ensured the long-term and successful preservation of the gene pool *ex situ-in vivo*, with the primary goal of stopping 'genetic erosion' and preserving local indigenous varieties (Avramov *et al.*, 1997). The first gene bank for the genus *Vitis* is located at the experimental agricultural farm Radmilovac, was established in 1960 and is run by the University of Belgrade's Faculty of Agriculture (Avramov and Jelenković, 1960). A total of 363 samples were collected - including varieties, species and vine rootstocks - and characterised and evaluated based on 84 descriptors between 1991 and 1993 (Avramov *et al.*, 1993). Today, there are three major ampelographic collections for the *Vitis* genus in the Serbian plant gene bank: i) Sremski Karlovci, an experimental vineyard within the University of Novi Sad's Faculty of Agriculture containing a total of 737 samples, ii) Radmilovac, an experimental vineyard within the University of Belgrade's Faculty of Agriculture containing a total of 659 samples, and iii) The Centre for Viticulture and Wine Production at Niš containing a total of 336 samples (Nikolić *et al.*, 2021). Results obtained by several authors (Rakonjac *et al.*, 2014; Štajner *et al.*, 2014) have confirmed high levels of diversity among cultivated varieties.

According to Aradhya *et al.* (2003), the germplasm of cultivated grapevines represents a unique and complex gene pool, with its structure determined by artificial selection and its vegetative manner by grapevine propagation. It has been confirmed that grapevine diversity, especially for *Vitis vinifera* cultivars, can be determined via different levels of molecular markers. Microsatellites, or simple repeated sequences (SSRs), have proven to be the most effective markers for grapevine genotyping (Laucou *et al.*, 2011; Jakše *et al.*, 2013), having properties which allow them to be widely used - from variety identification to parent reconstruction and genome mapping (Sefc *et al.*, 2001, Štajner, 2014).

Thomas and Scott (1993) were the first to use microsatellites for identifying grapevine varieties, showing them to be sequences which are ubiquitously present in the grapevine genome, thus providing a plethora of information necessary for identifying *Vitis vinifera* cultivars. Since many research groups around the world have become interested in the microsatellite genotyping of vines, a large number of these markers have been developed (Bowers *et al.*, 1996; Bowers *et al.*, 1999; Sefc *et al.*, 1999; Adam-Blondon *et al.*, 2004; Arroyo-Garcia and Martinez-Zapater, 2004; Di Gaspero *et al.*, 2005; Merdinoglu *et al.*, 2005; Goto-Yamamoto *et al.*, 2006).

TABLE 1. Investigated grapevine varieties and their basic characteristics.

Variety	Mean use	Colour of		Type of flower
		Skin	Flesh	
Alicante Henri Bouschet	W	N	S	Hermaphrodite
Babić veliki	W	N		Hermaphrodite
Blaufraenkisch	W/T	N		Hermaphrodite
Braghina rosie	W/T	Rs		Female
Bratkovina crna	W/T	N		Female
Cabernet-Sauvignon	W	N		Hermaphrodite
Cabernet-Sauvignon clon 10/32	W	N		Hermaphrodite
Cabernet-Sauvignon clon Radmilovac	W	N		Hermaphrodite
Cabernet franc clon 21/20	W	N		Hermaphrodite
Cot	W/T	N		Hermaphrodite
Dinka mirisava	W	Rg		Hermaphrodite
Gamay tenturier	W	N	S	Hermaphrodite
Lasina	W/T	N		Hermaphrodite
Kadarun	W	N		Hermaphrodite
Kadarka kek	W	N		Hermaphrodite
Koevidinka	W	Rs		Hermaphrodite
Krajinski bojadiser	W	N	S	Hermaphrodite
Noir hâtif de Marseille	W	N		Hermaphrodite
Pamid	W	Rs		Hermaphrodite
Piccola nera	W	Rs		Hermaphrodite
Pinot noir clon 658-12	W	N		Hermaphrodite
Plavina velika	W	N		Hermaphrodite
Plavina mala	W	N		Hermaphrodite
Prokupac	W	N		Hermaphrodite
Ruby Cabernet	W/T	N		Hermaphrodite
Rudežuša crna	W/T	N		Hermaphrodite
Srpski rubin	W	N		Hermaphrodite
Stanušina crna	W	N		Hermaphrodite
Vranac	W	N		Hermaphrodite
Župski bojadiser	W	N	S	Hermaphrodite

Mean use: Wine/Table; Colour of the berry epidermis: B = green-yellow; Rs = pink, rose; G = grey; N = dark blue; Rg = red; S = coloured mesocarp.

A defined set of six (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG7) or nine (the previous six combined with VVMD32, VVMD36 and VVMD25) highly polymorphic microsatellite markers is commonly used in grapevine genotyping studies, usually with the purpose of determining genetic variability between European grape varieties, which are highly polymorphic (Sefc *et al.*, 2001; Žulj Mihaljević *et al.*, 2013). The purpose of this study was to carry out the ampelographic characterisation, evaluation and microsatellite profiling of 30 vine varieties to find potential synonyms within this group, as well as to compare the obtained profiles with the available DNA profiles of grapevines from other regions in Europe.

MATERIALS AND METHODS

1. Ampelographic description

The examined material for this study came from the ampelographic collections at the University of Belgrade, Faculty of Agriculture's experimental vineyard Radmilovac. According to the regionalisation conducted in 2015, the Radmilovac vineyard belongs to the Belgrade region, Gročansko vinogorje (Ivanišević *et al.,* 2015). The geographical position of the collection is located at 44°45'24.66"N, 20°34'54.50"E. The vineyard is arranged rectangularly, 3 × 1 m, and the training system is an asymmetrical cordon with mixed pruning. Ampelographic characterisation was performed on 30 varieties that belong to the noble *Vitis vinifera* L. (Table 1). Forty-five characteristics were monitored during two consecutive vegetation periods in 2016 and 2017 (OIV, 2009, Cost action FA1003-GRAPENET). The most important ampelographic characteristics to be monitored were the morphological characteristics of young shoots, young leaves, shoots, flowers, mature leaves, grapes and berries and grape yield per m^2.

2. Extraction of DNA

For the extraction of total DNA, 150 mg of young fresh leaf tissue from the examined varieties was crushed to a fine powder with liquid nitrogen (Messer Tehnogas). Total DNA extraction was performed according to the 'ZR Plant/Seed DNA MiniPrep (USA)' protocol.

3. Measuring DNA concentration

DNA concentration was measured by spectrophotometry using 'Implen NanoPhotometer P300'. After determining the concentration, the samples were stored at −20 C until further analysis.

4. PCR amplification of microsatellites and capillary electrophoresis

A PCR reaction of microsatellite DNA chain amplification ('Polymerase Chain Reaction – PCR') was conducted as described by Štajner *et al*. (2011). The PCR mix was prepared in a total volume of 15 µl containing 20 ng of genomic DNA, 5× PCR buffer (Promega), 0.2 mM each of dNTPs (Sigma), 2 mM $MgCl_2$ (Promega), 0.5 U of GoTaq® DNA Polymerase (Promega), and three different primers – 2 pmol of each reverse and forward primer, and 2.5 pmol of fluorescently labelled M13 (−21) tail primer (5′-TGTAAAACGACGGCCAGT-3′). The tail primer was labelled with 6-FAM, VIC, PET or NED fluorescent dye. The shortest locus specific primer was elongated for the TAIL sequence at the 5′ end, which allowed economic fluorescent labelling of PCR products and enabled visualisation of the amplified DNA fragments by capillary electrophoresis, allowing fluorescence detection (Schuelke, 2000). 8 microsatellite loci (VVS2, VVMD7, VVMD27, VrZAG62, VrZAG7, VVMD32, VVMD36 and VVMD25) were amplified using the following thermal profile: initial denaturation at 95 C for 2 min, followed by five touchdown cycles at 94 C for 30 s; 60–1.0 C/cycle for 45 s and 72 °C for 1min 30s, followed by 30 cycles at 94 °C for 30 s; 55 °C for 45 s and 72 °C for 1 min 30 s; and a final step of 8 min at 72 °C. The cycling profile included touchdown steps in order to improve primer binding specificity. Differing fluorescent dye PCR reactions were merged together by aliquoting 4 µl of each. One microliter of merged PCRs was added to 0.5 µl of LIZ 600 size standard and 8.5 µl of Hi-Di formamide. Separation and visualisation of the PCR products was conducted in the laboratory of the University of Ljubljana's Biotechnical Faculty using the capillary sequencer 'ABI 3130XL Genetic Analyzer' (Applied Biosystems, US).

5. Data analysis

Amplified alleles were analysed and sized with GeneMapper software version 4.0 (Applied Biosystems, US). Genetic distances using the simple matching coefficient were calculated using DARwin 6.0.14 software (Leigh and Bryant, 2015) and used to draw a tree based on the weighted neighbour-joining clustering method, supported by bootstrap analysis.

The number of alleles per locus (No), the observed and expected heterozygosity (H_o and H_e), the polymorphic information content (PIC) and the frequency of null alleles (F_{null}) were calculated with Cervus 3.0 software (Kalinowski *et al.,* 2007). The identity analysis based on comparison among alleles of different studies/databases was performed with Cervus 3.0 software after standardisation of allele sizes using reference cultivars.

RESULTS AND DISCUSSION

The number of alleles per locus ranged from 4 (VVMD25) to 12 (VVMD28 and ZAG62), with a mean of 9 alleles, revealing a high level of variability in the sample set. The observed heterozygosity value (Ho) ranged from 0.64 (VVMD32 and VVMd7) to 0.85 (ZAG62) with a mean of 0.75, while the expected heterozygosity (He) ranged from 0.64 (VVMD25) to 0.90 (VVMD28) with a mean of 0.80. The observed heterozygosity showed higher values than the expected heterozygosity across two loci (VVS2 and VVMD25), and a slightly lower value than the expected heterozygosity for 6 loci out of 8. This observed heterozygosity deficiency may be related to the presence of null alleles, whose frequency values were positive for 5 of these loci (Table 2). The PIC (polymorphic information content) ranged from 0.58 (VVMD25) to 0.88 (VVMD28), with an average of 0.76. The loci with high PIC values (> 0.5) are classified as highly informative (Table 2).

The results of the ampelographic description (OIV codes) analysis are presented in Table 3 and the molecular characterisation in Table 4. While the examined varieties exhibited the same values for some ampelographic traits, differences were found in certain characteristics. The same assessment of all varieties was obtained for codes OIV 016 and OIV 241. For codes OIV 080, OIV 081-1*, OIV 081-2*, OIV 083-2*, OIV 151, OIV 209, OIV 220, OIV 221, OIV 235, OIV 236 and OIV 503, only two assessments/categories for the examined varieties were determined. For all other OIV codes, three or more categories were established for the examined varieties, which indicates greater divergences for the given traits.

The dendrogram shown in Figure 1 is based on ampelographic characteristics and shows three groups, comprising approximately the same number of varieties within each group. Group A comprises 10 varieties, with 4 subgroups. The first subgroup within group A consists of the following varieties: Župski bojadiser, Alicante Henri Bouschet and Prokupac. Out of a total of 45 descriptors, Župski bojadiser and Alicante Henri Bouschet share 32 similar characteristics. The similarities between Župski bojadiser (Alicante Henri Bouschet × Gamay noir) (Sivčev and Žunić, 2001) and Alicante Henri Bouschet (Petit Bouschet × Grenache) (Cabezas *et al.*, 2003) are explained by the fact that Alicante Henri Bouschet is the 'mother variety'. They are joined by Prokupac with 22 similar characteristics referring to young shoots. Differences can be perceived in the characteristics of young leaves and, when it comes to mature leaves, in the number of clippings in the anthocyanin pigment on the front of the leaf, the cross section shape of the mature leaf, the shape of the margin teeth,

TABLE 2. Statistical analysis of 8 SSR markers evaluated in 30 grapevine genotypes.

Locus	No	Ho	He	PIC	F_{null}	PI
VVMD28	12	0.77	0.90	0.88	0.07	0.02
ZAG79	10	0.82	0.88	0.85	0.02	0.03
ZAG62	12	0.85	0.86	0.82	-0.02	0.04
VVMD32	9	0.64	0.85	0.82	0.12	0.05
VVMD27	8	0.76	0.80	0.76	0.03	0.07
VVS2	11	0.80	0.79	0.75	-0.01	0.07
VVMD7	9	0.64	0.71	0.65	0.04	0.14
VVMD25	4	0.68	0.64	0.58	-0.04	0.19
Mean	**9**	**0.75**	**0.80**	**0.76**	**-**	***2.1x10 $^{-10}$**

No = number of alleles. Ho = observed heterozygosity. He = expected heterozygosity. PIC = polymorphic information content. Fnull = estimated frequency of null alleles and PI = probability of identity; *cumulative PI.

TABLE 3. Ampelographic characteristics of investigated grapevine varieties (Part 1/3).

Variety	OIV 001	OIV 003	OIV 004	OIV 006	OIV 007	OIV 008	OIV 016	OIV 051	OIV 053	OIV 067	OIV 068	OIV 070	OIV 072	OIV 074	OIV 075	OIV 076	OIV 079
Alicante Henri Bouschet	5	3	7	7	1	2	1	1	7	3	2	1	1	4	1	3	3
Babić veliki	5	3	3	3	1	1	1	1	3	4	3	1	5	3	3	4	7
Braghina rosie	5	3	7	3	3	1	1	4	7	3	3	3	5	5	1	4	3
Bratkovina crna	5	5	3	3	1	2	1	2	3	3	3	3	5	1	5	3	3
Cabernet franc clon 21/20	5	3	3	3	1	1	1	3	3	3	3	3	3	1	3	2	5
Cabernet-Sauvignon	5	7	5	1	1	1	1	3	3	4	4	3	3	1	3	3	3
Cabernet-Sauvignon clon 10/32	5	7	5	1	1	1	1	3	3	4	4	3	3	1	1	3	3
Cabernet-Sauvignon clon Radmilovac	5	5	7	1	1	1	1	3	3	4	4	1	3	1	3	3	3
Koevidinka	5	5	5	1	2	3	1	1	1	2	1	3	5	1	5	2	3
Dinka mirisava	5	3	3	1	1	3	1	1	1	4	1	3	5	5	3	3	3
Blaufraenkisch	5	3	3	1	1	2	1	1	1	3	1	2	7	2	5	3	3
Gamay tenturier	5	7	3	3	3	2	1	3	3	3	3	3	1	1	1	3	3
Kadarun	5	3	5	5	3	1	1	2	5	3	3	3	7	5	3	3	7
Krajinski bojadiser	5	7	3	3	3	2	1	3	3	3	3	3	1	1	5	3	3
Lasina	5	3	3	3	2	1	1	2	3	3	3	2	1	2	1	3	3
Cot	5	5	3	3	1	2	1	3	3	4	4	3	3	1	3	3	3
Noir hâtif de Marseille	5	5	3	1	1	2	1	1	1	3	3	2	7	3	3	2	3
Piccola nera	5	3	3	3	1	2	1	2	3	3	3	1	1	5	3	4	7
Pinot noir clon 658-12	5	3	3	1	1	1	1	1	1	3	2	1	1	1	5	3	3
Plavina mala	5	3	5	5	3	1	1	3	5	3	3	2	1	1	1	4	7
Plavina velika	5	3	7	7	3	1	1	2	7	4	4	3	3	5	3	4	7
Pamid	5	3	3	3	1	1	1	3	3	3	3	3	5	5	5	4	5
Prokupac	5	5	7	7	2	1	1	2	7	3	2	3	1	2	7	3	3
Ruby Cabernet	5	5	7	3	1	2	1	3	7	3	3	2	7	5	7	3	3
Rudežuša crna	5	3	3	3	3	1	1	1	3	3	1	1	5	5	3	3	3
Kadarka kek	5	3	3	3	1	2	1	3	3	3	3	2	5	5	5	4	3
Srpski rubin	5	5	3	3	1	2	1	1	3	3	3	1	9	2	3	3	3
Stanušina crna	5	3	3	3	1	1	1	1	3	2	2	2	1	2	5	2	3
Vranac	5	3	3	3	1	2	1	2	3	3	3	2	1	1	1	3	5
Župski bojadiser	5	7	7	7	3	2	1	2	7	3	3	3	1	2	3	2	3

TABLE 3. Ampelographic characteristics of investigated grapevine varieties (Part 2/3).

Variety	OIV 80	OIV 081-1*	OIV 082-1*	OIV 083-2*	OIV 84	OIV 87	OIV 94	OIV 151	OIV 155	OIV 202	OIV 204	OIV 206	OIV 208	OIV 209	OIV 220
Alicante Henri Bouschet	1	1	1	1	5	5	3	3	9	5	5	5	2	3	3
Babić veliki	1	1	1	1	1	3	3	3	9	5	3	3	2	3	3
Braghina rosie	3	1	1	1	5	5	5	4	5	5	5	3	1	3	5
Bratkovina crna	1	1	1	1	3	3	9	4	9	5	5	5	2	3	3
Cabernet franc clon 21/20	1	1	1	9	1	3	3	3	3	5	5	5	3	3	3
Cabernet-Sauvignon	1	1	2	1	3	3	7	3	5	5	5	3	2	2	3
Cabernet-Sauvignon clon 10/32	1	1	2	1	3	3	5	3	5	3	5	3	2	2	3
Cabernet-Sauvignon clon Radmilovac	1	1	2	1	3	5	3	3	5	5	3	3	2	2	3
Koevidinka	1	1	1	9	5	5	3	3	9	5	3	3	2	3	3
Dinka mirisava	1	9	1	1	3	5	3	3	9	5	3	1	1	3	3
Blaufraenkisch	3	1	1	1	3	3	5	3	5	3	5	3	1	3	5
Gamay Tenturier	3	1	1	1	3	3	3	3	9	3	5	3	2	3	3
Kadarun	1	1	1	1	3	5	5	3	9	5	9	3	3	3	5
Krajinski bojadiser	1	1	1	9	3	3	3	3	9	5	5	3	1	3	5
Lasina	1	1	1	1	1	3	5	3	9	5	3	3	1	3	5
Cot	3	1	1	1	3	3	5	3	9	5	3	3	1	3	3
Noir hâtif de Marseille	1	1	1	1	1	3	3	3	5	5	5	3	1	3	3
Piccola nera	1	1	1	1	1	3	5	3	9	3	5	3	1	3	3
Pinot noir clon 658-12	1	1	1	1	5	1	3	3	9	3	7	3	1	2	3
Plavina mala	1	1	1	1	5	3	5	3	9	5	5	3	3	3	3
Plavina velika	1	1	1	1	5	3	5	3	9	5	5	5	3	3	5
Pamid	1	1	1	1	5	3	5	3	9	5	5	3	1	3	3
Prokupac	3	1	1	1	5	5	5	3	9	5	5	3	2	3	5
Ruby Cabernet	1	1	1	1	5	1	3	3	7	7	5	5	2	3	5
Rudežuša crna	1	1	1	1	3	3	1	3	9	5	5	3	1	3	5
Kadarka Kek	1	1	1	1	5	3	3	3	5	5	5	3	1	3	5
Srpski rubin	3	1	1	1	1	1	3	3	9	5	5	1	1	3	3
Stanušina crna	3	1	1	1	3	3	3	3	9	5	7	3	2	3	5
Vranac	1	1	1	1	3	1	7	3	5	5	3	1	2	2	3
Župski bojadiser	1	1	1	1	1	3	3	3	9	5	5	5	1	3	3

TABLE 3. Ampelographic characteristics of investigated grapevine varieties (Part 3/3).

Variety	OIV 221	OIV 223	OIV 225	OIV 231	OIV 235	OIV 236	OIV 241	OIV 301	OIV 303	OIV 351	OIV 502	OIV 503	OIV 504
Alicante Henri Bouschet	5	2	6	7	1	1	3	3	5	3	5	3	5
Babić veliki	3	4	6	3	1	1	3	3	5	3	3	3	3
Braghina rosie	3	2	2	1	1	4	3	3	5	5	3	3	3
Bratkovina crna	3	2	6	1	1	1	3	3	5	3	3	3	3
Cabernet franc clon 21/20	3	2	6	1	1	1	3	5	7	3	3	3	3
Cabernet-Sauvignon	3	2	6	1	1	4	3	5	7	3	3	3	1
Cabernet-Sauvignon clon 10/32	5	2	6	1	1	4	3	5	5	3	3	3	1
Cabernet-Sauvignon clon Radmilovac	3	2	6	1	1	4	3	5	5	3	3	3	3
Koevidinka	3	2	2	1	1	4	3	5	5	3	3	3	7
Dinka mirisava	5	2	3	1	1	1	3	5	5	3	3	3	5
Blaufraenkisch	3	2	6	1	1	1	3	3	5	5	3	3	3
Gamay Tenturier	3	2	6	7	1	4	3	3	3	5	3	3	3
Kadarun	3	2	6	1	1	1	3	3	3	5	3	3	9
Krajinski bojadiser	3	2	6	7	1	1	3	5	5	3	3	5	7
Lasina	3	3	6	1	1	4	3	3	5	5	3	3	3
Cot	3	2	6	3	1	4	3	3	5	3	3	3	5
Noir Hatif de Marseille	3	3	6	1	1	1	3	3	3	3	3	3	3
Piccola nera	3	2	6	3	1	1	3	3	5	5	3	3	5
Pinot noir clon 658-12	3	3	6	1	1	4	3	3	3	5	3	3	3
Plavina mala	3	2	6	1	1	1	3	3	5	3	3	3	3
Plavina veliki	3	2	6	1	1	1	3	3	5	5	3	3	5
Pamid	3	4	5	1	1	4	3	3	5	7	3	3	3
Prokupac	3	2	6	1	2	1	3	3	7	5	3	3	9
Ruby Cabernet	3	5	5	1	2	1	3	5	5	3	5	3	3
Rudežuša crna	3	2	6	1	1	1	3	1	5	3	3	3	5
Kadarka Kek	3	2	6	1	1	1	3	3	5	3	3	3	7
Srpski rubin	3	3	6	1	1	4	3	5	5	5	3	3	5
Stanušina crna	3	4	6	1	1	4	3	3	5	3	3	3	7
Vranac	3	3	6	1	1	4	3	3	5	5	7	3	7
Župski bojadiser	3	2	6	7	1	1	3	3	5	3	3	3	5

TABLE 4. Allelic profiles of investigated grapevine varieties analysed at 8 microsatellite loci.

Individual ID	VVS2	VVS2	VVMD28	VVMD28	VVMD7	VVMD7	ZAG79	ZAG79	VICVVMD27	VICVVMD27	VVMD25	VVMD25	VVMD32	VVMD32	ZAG62	ZAG62
Alicante Henry Bouschet	139	141	235	247	259	273	260	264	194	197	259	273	266	266	205	205
Babić veliki	139	150	243	247	255	265	272	272	194	194	257	259	266	278	207	207
Blaufraenkische	/	/	233	245	255	265	250	250	194	210	267	267	/	/	211	222
Braghina rosie	129	131	233	233	255	263	250	272	197	197	257	257	272	286	205	222
Bratkovina crna	/	/	245	245	255	255	256	264	194	197	257	267	284	286	205	214
Cabernet franc clon 21/20	135	143	227	234	255	279	260	272	197	205	257	273	253	272	211	222
Cabernet-Sauvignon	135	147	233	235	255	255	260	260	191	205	257	267	254	254	205	211
Cabernet-Sauvignon clon 10/32	/	/	233	233	/	/	/	/	190	197	257	257	/	/	/	/
Cabernet-Sauvignon clon Radmilovac	/	/	233	235	255	255	260	260	191	205	257	267	253	253	205	212
Koevidinka	129	129	/	/	255	265	256	264	197	205	257	267	253	253	211	222
Dinka mirisava	129	139	235	244	255	257	264	268	194	194	257	259	278	286	204	205
Gamay tenturier	141	145	243	243	255	265	252	270	201	210	259	267	264	286	204	205
Krajinski bojadiser	141	145	243	243	255	273	252	270	201	210	259	267	264	264	203	205
Lasina	129	129	235	244	249	255	250	262	197	197	257	257	/	/	214	214
Cot	129	147	233	266	255	279	258	272	205	207	257	267	254	266	205	220
Noir hâtif de Marseille	129	133	217	256	259	265	258	268	194	205	257	259	266	286	204	205
Piccola nera	129	129	/	/	255	265	256	272	197	197	257	257	270	286	214	222
Pinot noir clon 658-12	133	148	217	235	255	259	252	258	201	205	257	267	254	286	205	211
Plavina mala	129	139	247	256	265	265	250	256	194	205	257	257	266	278	206	218
Plavina velika	129	139	247	256	265	265	250	256	194	205	257	257	278	278	205	211
Pamid	129	129	244	244	255	255	256	264	194	197	257	257	286	286	206	214
Prokupac	139	141	244	258	265	265	256	264	197	201	259	273	286	286	211	218
Rubi Cabernet	129	131	/	/	255	255	256	258	194	201	/	/	/	/	/	/
Rudežuša crna	129	139	227	233	255	269	264	272	197	197	257	273	264	266	205	218
Kadarka Kek	129	131	233	258	265	269	256	272	201	205	257	273	266	286	209	218
Srpski rubin	129	139	245	258	265	265	256	264	197	201	257	273	253	286	211	211
Stanušina crna	129	131	235	243	255	265	256	272	197	201	257	257	264	286	205	212
Vranac	129	129	235	247	263	265	272	272	197	197	257	259	270	270	211	218
Župski bojadiser	133	141	217	258	265	265	258	270	205	210	257	259	264	286	205	222

/Not amplified.

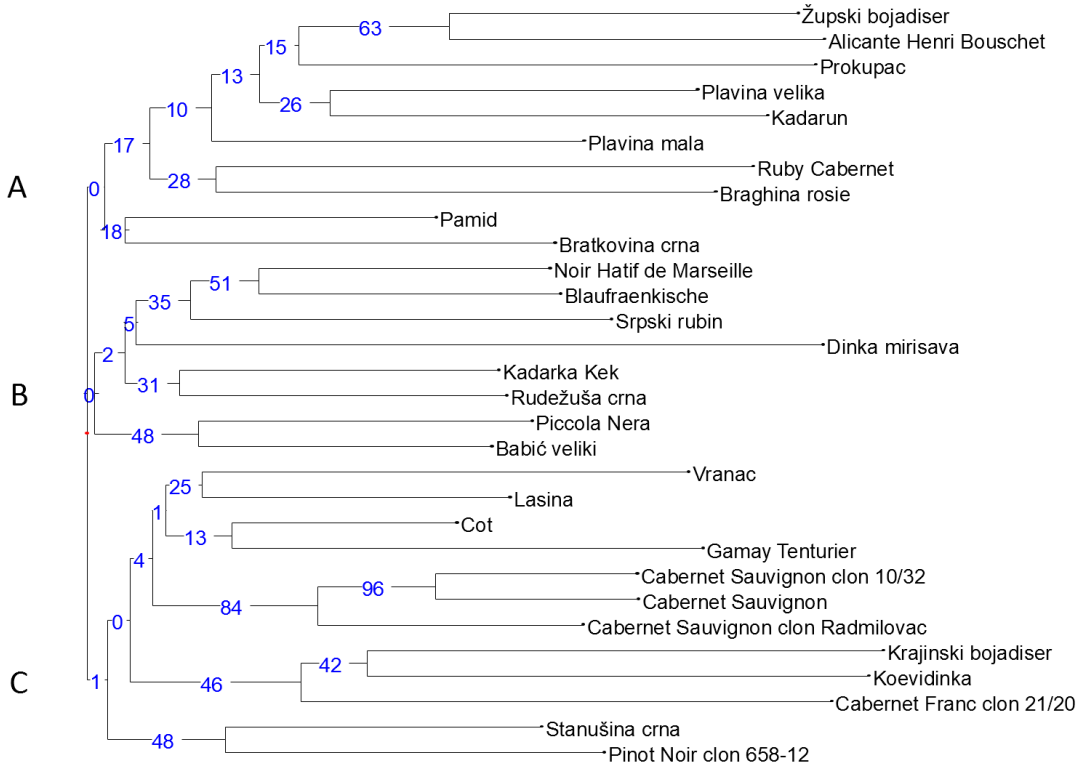

FIGURE 1. Dendrogram of ampelographic characteristics of the investigated grapevine varieties.

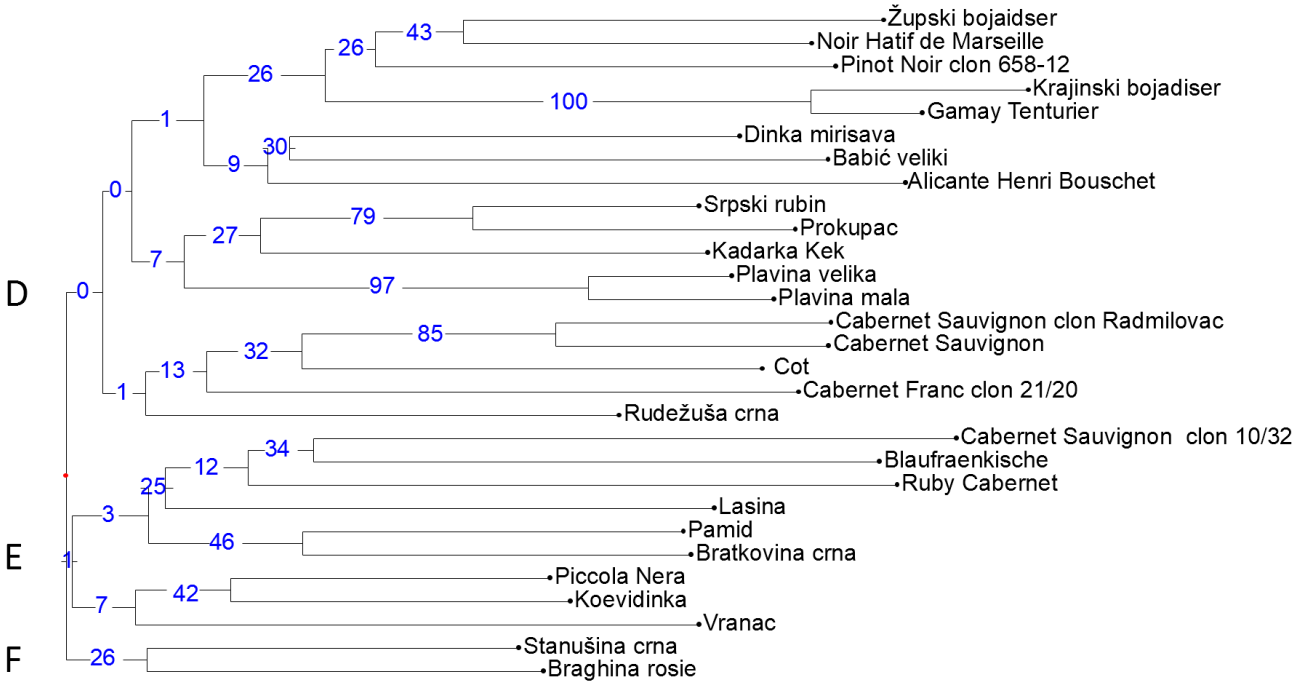

FIGURE 2. Dendrogram based on the SSR markers of the investigated grapevine varieties, using the simple matching dissimilarity coefficient and the weighted neighbour-joining clustering method. The numbers on the branches indicate the percentage of bootstrap values (1000).

the shape of the base petiole sinus, upright density, the lying hairs of the mature leaf, the length of the petiole, the shape of the cluster, the length and width of the berry, the anthocyanin pigment and firmness of the berry flesh, the phenology and yield per m^2.

In the second subgroup, the Plavina velika variety is more similar to the Kadarun variety than to the Plavina mala variety. The differences between Plavina velika and Plavina mala can be detected in the characteristics of the young shoots (OIV 004, OIV 006), young leaves (OIV 051, OIV 053) and mature leaves (OIV 067, OIV 068, OIV 070, OIV 072, OIV 074, OIV 075). In the *V*IVC database (www.vivc.de), the Plavina crna variety is listed as a synonym of Plavina mala, and the origin of Plavina crna has been confirmed (Primitivo × Lagorthi) (Štajner *et al.*, 2015). The molecular analysis of this study, based on 8 microsatellite markers, also confirmed the same genetic profile for Plavina crna and Plavina mala. Our study resulted in in difference for one allele between the two genotypes, Plavina crna and Plavina mala (Table 5).

In the third subgroup, the varieties Ruby Cabernet (Carignan × Cabernet-Sauvignon) (https://worldsbestwines.eu/grapes/ruby-cabernet/) and Braghina rosie differ significantly in type of flower, but are similar across 21 characteristics. Based on the SSR markers, these two varieties belong to different groups (Table 3, Figure 2).

The last subgroup consists of the varieties Pamid and Bratkovina crna, which share 30 similar characteristics, but only Pamid can produce extremely high yields (OIV 351). The similarities were confirmed with SSR markers. These two varieties differ by only two out of 14 compared alleles, and form a subgroup within group E.

Group B unites 8 varieties, divided into three subgroups. In the first subgroup, Noir hâtif de Marseille and Blaufränkisch stand out, joined by the variety Srpski rubin. The variety Dinka mirisava differs significantly from the three aforementioned varieties, based on ampelographic characteristics (Figure 1). It is important to point out that Dinka mirisava is not in the *V*IVC database (www.vivc.de), but has been attached to this subgroup, as it can be significantly differentiated from the others by the colour of the young shoots, the characteristics of the mature leaf, clusters and berries, and its phenology. However, based on the SSR markers, Dinka mirisava and Noir hâtif de Marseille (Muscat Rouge de Madere × Pinot) are distant (Table 3, Figure 2). The first subgroup of varieties (Blaufränkisch, Noir hâtif de Marseille and Srpski rubin) within group B were created by spontaneous hybridisation, but there are significant deviations in the ampelographic characteristics of the shoot tips and the mature leaves. The second subgroup of group B consists of the varieties Kadarka Kek and Rudežuša crna. In the *V*IVC database (www.vivc.de), the primary name of the variety Skadarka is Kadarka Kek, originating in Hungary, while Rudežuša crna originates in the former Yugoslavia. These two varieties share 33 characteristics, but differ in young shoot colour, most leaf characteristics, basal bud fertility and phenology. The distance between the varieties of Kadarka Kek and Rudežuša crna was also confirmed with SSR markers (Table 4, Figure 2). The third subgroup within group B consists of the varieties Piccola nera and Babić veliki. They are similar in 33 characteristics, differing in the characteristics of young shoots, mature leaves, epidermis colour and fertility. In the *V*IVC database (www.vivc.de), one of the synonyms for Babić veliki is Babić crni, which is its primary name. Based on the SSR markers, Babić veliki and Vranac belong to different groups (Figure 2).

Group C consists of 12 varieties, with five subgroups. The first subgroup within group C consists of the varieties Vranac and Lasina, which share 31 similar ampelographic characteristics. Based on DNA analysis (i.e., the eight SSR markers), Vranac and Lasina belong to the same group (Figure 2). The second subgroup consists of the Côt and Gamay Tenturier varieties, which also share 31 similar characteristics. Differences were found in shoot colour, the back of the internode and mature leaf characteristics, as well as in phenology.

The third subgroup of group C consists of Cabernet-Sauvignon, Cabernet-Sauvignon clone 10/32 and Cabernet-Sauvignon clone Radmilovac, which share 33 characteristics and differ in young shoots, the back of the internode, the characteristics of the mature leaves and phenology. The fourth subgroup consists of the Cabernet franc clone 21/20, Koevidinka and Krajinski bojadiser (Gamay noir × Gamay Tenturier), which share 19 characteristics and differ in young shoots, mature leaves and phenology. The last subgroup in group C consists of the varieties Stanušina crna and Pinot noir clone 658-12. They are similar across 28 characteristics,

TABLE 5. Differences in SSR markers between varieties from this study and from other researchers.

Sample name/SSR loci	VVS2		VVMD7		VVMD25		VVMD27		VVMD28		VVMD32		Reference
KABERNE_SOVINJON_POPULACIJA	137	149	239	239	238	248	172	186	233	235	239	239	our data
Cabernet-Sauvignon (#322)	137	149	239	239	238	248	172	186	233	235	239	239	Lacombe *et al.* (2013)
VRANAC	131	131	247	249	238	240	178	178	235	247	255	255	our data
Vranac_BIH	131	131	247	247	238	240	178	178	235	247	255	255	Štajner *et al.* (2014)
Vranac_MNE	131	131	247	247	238	240	178	178	235	247	255	255	Štajner *et al.* (2014)
PIKOLA_NERA	131	131	239	249	238	238	178	178	/	/	255	271	our data
Plavina-maločrn	131	131	239	249	238	238	178	178	/	/	255	271	Štajner *et al.* (2014)
Maločrn	131	131	239	249	238	238	178	178	/	/	255	271	Štajner *et al*. (2014)
PROKUPAC	141	143	249	249	240	254	178	182	244	258	271	271	our data
Prokupac(#1630)	141	143	249	249	240	254	178	182	245	259	271	271	Lacombe *et al.* (2013)
Prokupac_BIH	141	143	249	249	240	254	178	182	245	259	271	271	Štajner *et al.* (2014)
STANUŠINA_CRNA	131	133	239	249	238	238	178	182	235	243	249	271	our data
STANUSINA_CRNA_RNM	131	133	239	249	238	238	178	182	235	243	249	271	*V*IVC database
PLAVINA VELIKA	131	141	249	249	238	238	175	186	247	256	263	263	our data
PLAVINA MALA	131	141	249	249	238	238	175	186	247	256	251	263	our data
Plavina Crna_CRO (Primitivo)	131	141	239	249	238	238	176	186	247	257	251	263	*V*IVC database
BAGRINA_UREZANOG_LISTA	131	133	239	247	238	238	178	178	233	233	257	271	our data
Bagrina_SRB	131	133	239	247	238	238	176	178	233	233	257	271	Štajner *et al.* (2014)
LASINA	131	131	233	239	238	238	178	178	235	244	/	/	our data
LASINA_CRO	131	131	233	239	238	238	176	178	235	245	239	255	*V*IVC database
Lasina(#1642)	131	131	233	239	238	238	176	178	235	245	239	255	Lacombe *et al.* (2013)
BABIĆ_VELIKI	141	152	239	249	238	240	175	175	243	247	251	263	our data
Babic_CRO	141	149	247	249	238	238	176	176	243	247	239	251	*V*IVC database
BabicBIH	141	149	247	249	238	238	176	176	243	247	239	251	Štajner *et al.* (2014)
BRATKOVINA_CRNA	/	/	239	239	238	248	175	178	245	245	269	271	our data
Bratkovina_crna_CRO	131	133	239	239	238	254	178	191	235	235	263	271	*V*IVC database
Bratkovina_crna(#1856)	131	133	239	239	238	254	178	191	235	235	263	271	Lacombe *et al.* (2013)
PLOVDINA_CRNA	131	131	239	239	238	238	175	178	244	244	271	271	our data
PlovdinaCrna_SRB	141	141	239	255	254	254	178	178	227	245	263	271	Štajner *et al*. (2014)
ALIKANT_BUŠE	141	143	243	257	240	254	175	178	235	247	251	251	our data
AlicanteHenriBouschet(#514)	131	143	239	243	240	240	178	191	243	259	249	271	Lacombe *et al.* (2013)
AlicanteHenriBouschet_FRA	131	143	239	243	240	240	178	191	243	259	249	271	*V*IVC database

/not amplified.

with differences in the young leaf (i.e., the pigment of the upper side of the front of the leaf – the fourth leaf), the cross-sectional shape of the mature leaf, the anthocyanin colouration of the main nerves on the front of the leaf, cluster and berry length and shape, phenology and yield per m^2. In the Kadarun variety, alleles were not collected from 8 loci, which means that the DNA was probably weak, so it was removed from the dendrogram. The VVMD5 locus was also rejected, as the amplification was very weak and, therefore, the alleles could not be 100 % identified.

According to Nastev (1967), Lisičina is the wrong synonym for the variety Plovdina (Pamid). In *V*IVC (www.vivc.de), only one variety was recorded under number *V*IVC 9557 and the name Plavina crna. The parents of Plavina crna were found to be the varieties Primitivo and Lagorthi. An important difference between the varieties Braghina rosie, Dinka crvena and Dinka mirisava was found to be in flower type: both varieties with the prefix 'dinka' have a hermaphrodite flower, while the Braghina rosie has a functionally female flower. In *V*IVC (www.vivc.de), Braghina rosie has 60 synonyms, including several containing 'dinka'. Pamid is a variety that is traditionally grown together with Prokupac in the same vineyards (Bešlić *et al.*, 2012). Prokupac has a long history of red wine production, but has been neglected for decades due to the introduction of international varieties known for their potential to produce high quality wines.

The dendrogram, which was created based on molecular markers (Figure 2), consists of three groups: group D, the most numerous with 18 varieties; group E with 9 varieties; and group F with only two varieties. Most of the mentioned varieties from these groups belong to the eco-geographical group *convar. occidentalis*.

From comparing ampelographic features (Figure 1) and molecular markers (Figure 2), it can be observed that there are three groups of varieties within each dendrogram. The similar number of varieties in each group is shown on an ampelographic dendrogram, and this concordance is based on 31-32 features out of a total of 45. Results from a two-year study by Garcia-Muñoz *et al.* (2011) showed that qualitative ampelographic characteristics manifested significant differences; namely, the characteristic OIV 051 (colouration of the upper side of the young leaf) significantly deviates in both years of testing in 27 monitored varieties. The varieties covered by this research, a total of 30, originate from several countries around the world. The results confirm a high level of diversity for this group, in accordance with previous research (Laucou *et al.*, 2011; Štajner *et al.*, 2014), which is most likely due to the trade routes that existed in the once unified state of Yugoslavia. Bešlić *et al.* (2012) came to similar conclusions. Bacillieri *et al.* (2013) reported the genetic structure of varieties with 2,096 genotypes and using 20 microsatellite markers; they showed that there are three main genetic groups of cultivated grapevine varieties related to nationality and geography – Western European, Balkan and Eastern European – and groups in which the table varieties of the Eastern Mediterranean, Caucasus, Middle East and Far East predominate. The combination of molecular and morphological characterisations has led to good management of grapevine genetic resources (Balda *et al.*, 2014; Maul and Töpfer, 2015; Ferreira *et al.*, 2015).

Identity analysis and comparison among microsatellite alleles for 6 loci was done based on datasets from Štajner *et al*. (2014), Lacombe *et al*. (2013) and VIVC database (Maul *et al*. 2021). The data in Table 5 show microsatellite alleles obtained in our analysis and those from other studies. Alleles of the same loci that differ by 1 bp are expected to be the same. Alleles from our analysis that differ from those obtained by other studies are marked in grey. For 5 groups of varieties (Cabernet, Vranac, Plavina, Prokupac and Stanusina) we confirmed identical allelic profiles in all compared loci. The genotypes Plavina velika, Plavina mala and Plavina crna that differ in 1-2 alleles can be considered as near synonyms. For the two groups of genotypes (Bagrina and Lasina) mutations resulting in difference of 2 bp for only 1 allele may be the consequence of clonal variation. Within group of genotypes Babić differences were observed in a few loci, but 1 allele of each loci was shared among genotypes, meaning that these genotypes may have a parent-offspring relationship. The samples called Bratkovina, Plovidna and Alicante are probably misnomers as they show different allelic profiles from reference data and their "true-to-type" identity was not confirmed.

CONCLUSION

Among the examined varieties, a large variability in ampelographic characteristics was found. The dendrogram was constructed based on the ampelographic characteristics of three groups, with approximately the same number of varieties within each group.

The dendrogram was created based on the molecular markers of the three groups, of which the first group – the most numerous – consisted of 18 varieties, the second group of nine varieties and third group of only two varieties.

The integration of the ampelographic data with the genetic data is of utmost importance for accurate identification of the varieties, offering a significant means for the preservation and use of the varieties. The integration of the ampelographic data with the genetic data is of utmost importance for the accurate identification of varieties, offering a significant means of variety preservation and use.

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REFERENCES

Adam-Blondon, A.F., Roux, C., Claux, D., Butterlin, G., Merdinoglu, D., & This, P. (2004). Mapping 245 SSR markers on the *Vitis vinifera* genome: a tool for grape genetics. *Theoretical and Applied Genetics*, 109(5), 1017-1024. https://doi.org/10.1007/s00122-004-1704-y

Alleweldt, G. (1988). The genetic resources of *Vitis.* World list of grapevine collection. 1st edition. *Institut für Rebenzüchtung Geiweilerhof-Siebeldingen, Germany*.

Aradhya, M.K., Dangl, G.S., Prin, B.H., Boursiquot, J.M., Walker, M.A., Meredith, C.P., & Simon, C.J. (2003). Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L. *Genetics Research,* 81, 179-192. https://doi.org/10.1017/S0016672303006177

Arroyo-Garcia, R., & Martinez-Zapater, J.M. (2004). Development and characterization of new microsatellite markers for grape. *Vitis*, 43(4), 175-178.

Avramov, L., & Jelenković, G. (1960). Formiranje centra za selekciju vinove loze. *Poljoprivreda*, *10/11/12*, 39-46.

Avramov, L., Pavlović, K., Sivčev, B., Gašić, N., & Žunić, D. (1993). Description ampelographique du 363 cepages spec. *Vitis*. Ofice International de la Vigne et du Vin. Paris, 1-370.

Avramov, L., Žunić, D., Sivčev, B., Gašić, N., Živković, T., & Pandilović, S. (1997). Genofond sejanaca vinove loze poljoprivrednog fakulteta u Zemunu. *Savremena Poljoprivreda*, *XX* (3-4), 99-105.

Balda, P., Ibáñez, J., Sancha, J.C., & Martínez de Toda, F. (2014). Characterization and identification of minority red grape varieties recovered in Rioja, Spain. *American Journal of Enology and Viticulture,* 65, 148-152. https://doi.org/10.5344/ajev.2013.13050

Bešlić, Z., Todić, S., Korać, N., Lorenz, F., Emanuelli, M., & Grando, S. (2012). Genetic characterization and relationships of traditional grape cultivars from Serbia. *Vitis, 51*, 183-189.

Bowers, J.E., Vignani, R., & Meredith, C.P. (1996). Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome*, 39, 628-633. https://doi.org/10.1139/g96-080

Bowers, J.E., Dangl, G.S., & Meredith, C.P. (1999). Development and characterization of additional microsatellite DNA markers for grape. *American Journal of Enology and Viticulture*, *50*, 243-246.

Cabezas, J.A., Cervera, M.T., Arroyo-García, R., Ibáñez, J., Rodríguez-Torres, I., Borrego, J., Cabello, F., & Martínez-Zapater, J.M. (2003). Garnacha and Garnacha Tintorera: Genetic Relationships and the Origin of Teinturier Varieties Cultivated in Spain. *American Journal of Enology and Viticulture*. 54. 237-245.

Cindrić, P., Avramov, L., Korać, N., & Pejović, Lj. (1997). Genetički resursi vinove loze Jugoslavije. *Savremena Poljoprivreda*, 46(1-2), 175-184.

Di Gaspero, G., Cipriani, G., Marrazzo, M.T., Andreetta, D., Castro, M.J.P., Peterlunger, E., & Testolin, R. (2005). Isolation of (AC)n-microsatellites in *Vitis vinifera* L and analysis of genetic background in grapevines under marker assisted selection. *Molecular Breeding,* 15, 11-20. https://doi.org/10.1007/s11032-004-1362-4

Ferreira, V., Pinto-Carnide, O., Mota, T., Martín, J.P., Ortiz, J.M., & Castro, I. (2015). Identification of minority grapevine cultivars from Vinhos Verdes Portuguese DOC Region. *Vitis*, 54, 53-58.

Garcia-Muñoz, S., Muñoz-Organero, G., & Andrés María de Teresa, F.C. (2011). Ampelography – An old technique with future uses: The case of minor varieties of *Vitis vinifera* L. from the Balearic Islands. *Journal International des Sciences de la Vigne et du Vin*, 45(3), 125-137. https://doi.org/10.20870/oeno-one.2011.45.3.1497

Goto-Yamamoto, N., Mouri, H., Azumi, M., & Edwards, K.J. (2006). Development of grape microsatellite markers and microsatellite analysis including oriental cultivars. *American Journal of Enology and Viticulture*, *57*, 105-108.

Ivanišević, D., Jakšić, D., & Korać, N. (2015). Popis poljoprivrede (2012). *Poljoprivreda u Republici Srbiji. Vinogradarski atlas*. Republički zavod za statistiku, Beograd.

Jakšić, D., Ivanišević, D., Đokić, V., & Brbaklić, Tepavac, M. (2015). *Vinski atlas*. Republički zavod za statistiku, Beograd.

Jakše, J., Štajner, N., Tomić, L., & Javornik, B. (2013). Application of microsatellite markers in grapevine and olives. In: B. Sladonja & D. Poljuha (Eds.), *The Mediterranean Genetic Code – Grapevine and Olive*, 25-41. IntechOpen. https://doi.org/10.5772/53411

Kalinowski, S., Taper, M., & Marshall, T. (2007): Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.*, 16, 1099-1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Lacombe, T., Boursiquot, J.-M., Laucou, V., Di Vecchi-Staraz, M., Peros, J.-P., & This, P. (2013). Large-scale parentage analysis in an extended set of grapevine cultivars (*Vitis vinifera* L.). *Theor. Appl. Genet.* 126, 401-414. https://doi.org/10.1007/s00122-012-1988-2

Laucou, V., Lacombe, T., Dechesne, F., Siret, R., Bruno, J.P., Dessup, M., Dessup, T., Ortigosa, P., Parra, P., Roux, C., Santoni, S., Varès, D., Péros, J.P., Boursiquot, J.M., & This, P. (2011). High throughput analysis of grape genetic diversity as a tool for germplasm collection management. *Theoretical and Applied Genetics*, 122(6), 1233-1245. https://doi.org/10.1007/s00122-010-1527-y

Leigh, J., & Bryant, D. (2015). Popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6, 1110-1116. https://doi.org/10.1111/2041-210X.12410

Maul *et al*. (2021): *Vitis* International Variety Catalogue, www.vivc.de

Maul, E., & Töpfer, R. (2015). *Vitis* International Variety Catalogue (*V*IVC). A cultivar database references by genetic profiles and morphology. *BIO Web of Conferences*, 5, 01009. https://doi.org/10.1051/bioconf/20150501009

Maul, E., Sudharma, K.N., Ganesh, A., Hundemer, M., Kecke, S. Marx, G. Schreiber, T., Walk, M., vom Weg, S., Mahler-Ries, A., Brühl, U., & Töpfer, R. (2014). 30 Years *V*IVC - *Vitis* International Variety Catalogue (www.vivc.de). XI International Conference on Grapevine Breeding and Genetics, Yanqing, Beijing, China, July 28 - August 2, 2014

Merdinoglu, D., Butterlin, G., Bevilacqua, L., Chiquet, V., Adam-Blondon, A.F., & Decroocq, S. (2005). Development and characterization of a large set of microsatellite markers in grapevine (*Vitis vinifera* L.) suitable for multiplex PCR. *Molecular Breeding*, 15(4), 349-366. https://doi.org/10.1007/s11032-004-7651-0

Milutinović, M., Nikolić, D., Avramov, L., & Rakonjac, V. (2000). Recombination of some characteristics in F_1 generation of grapevine. *Acta Horticulturae*, 528(2), 641-644. https://doi.org/10.17660/ActaHortic.2000.528.95

Nastev, D. (1967). Specijano Lozarstvo (Particular viticulture). Izdanie na Universitetot vo Skopje, Skopje.

Nikolić, D., Ranković-Vasić, Z., & Atanacković, Z. (2015). New Serbian grapevine genotypes for red wine production. *Vitis*, 54, 165-168.

Nikolić, D., Miljković, J., Rakonjac, V., Radojević, I., & Ranković-Vasić, Z. (2018a). Inheritance and phenotypic correlations of agronomic traits in grapevine offsprings. *Acta Scientiarum Polonorum Hortorum Cultus*, 17(5), 87-99. https://doi.org/10.24326/asphc.2018.5.8

Nikolić, D., Banjanin, T., & Ranković-Vasić, Z. (2018b). Variability and heredity of some qualitative and quantitative grapevine characteristics. *Genetika*, 50(2), 549-560. https://doi.org/10.2298/GENSR1802549N

Nikolić, D., Korać, N., Todić, S., Jakšić, D., & Ivanišević, D. (2021). Grapevine genetic diversity in Serbia. First International Conference on Vranac and other Montenegrin Indigenous Sorts of Grapevine, Podgorica, Montenegro, 20-22 November (2017, Montenegrin Academy of Sciences and Arts, Scientific Meetings Volume 159, The Section of Natural Sciences Volume 22, 157-172.

OIV (2009). OIV Descriptor list for grape varieties and *Vitis* species, 2nd edition. Organisation Internationale de la Vigne et du Vin, Paris, France.

Rakonjac, V., Korać, N., Todić, S., Medić, M., Beslić, Z., Kuljanić, I., Ivanisević, D., & Popov, M. (2014). Genetic diversity of a Serbian grapevine germplasm collection based on morphoagronomic characteristics. *Genetika*, 46(3), 719-730. https://doi.org/10.2298/GENSR1403719R

Regner, F., Hack, R., & Eisenheld, C. (2004). Traditional and innovative grapevine breeding. *Bulletin OIV,* 77(885-886), 811-819.

Schuelke, M. (2000). An economic method for the fluorescent labelling of PCR fragments. Nat. Biotechnol., 18, 233-234. https://doi.org/10.1038/72708

Sefc, K.M., Regner, F., Turetschek, E., Glössl, J., & Steinkellner, H. (1999). Identification of microsatellite sequences in *Vitis riparia* and the irapplicability for genotyping of different *Vitis* species. *Genome*, 42, 367-373. https://doi.org/10.1139/g98-168

Sefc, K.M., Lefort, F., Grando, M.S., Scott, K., Steinkellner, H., & Thomas, M.R. (2001). Microsatellite markers for grapevine: A state of the art. In: K.A. Roubelakis-Angelakis (Ed.), *Molecular Biology and Biotechnology of Grapevine* (pp. 407–438). Amsterdam, Kluwer Publishers. https://doi.org/10.1007/978-94-017-2308-4_17

Sivčev, B., & Žunić, D. (2001). Ampelographic characteristics of new wine varieties created at the experimental station 'Radmilovac'. *Journal of Agricultural Sciences*, *46*(2), 105-116.

Štajner, N., Rusjan, D., Korošec-Koruza, Z., & Javornik, B. (2011). Genetic characterization of old Slovenian grapevine varieties of *Vitis vinifera* L. by microsatellite genotyping. *American Journal of Enology and Viticulture*, *62*(2), 250-255. https://doi.org/10.5344/ajev.2011.10011

Štajner, N., Tomić, L., Ivanišević, D., Korać, N., Cvetković-Jovanović, T., Beleski, K., Angelova, E., Maraš, V., & Javornik, B. (2014). Microsatellite

inferred genetic diversity and structure of Western Balkan grapevines (*Vitis vinifera* L.), *Tree Genetics & Genomes*, *10*, 127-140. https://doi.org/10.1007/s11295-013-0670-4

Štajner, N., Tomić, L., Progar, V., Pokorn, T., Lacombe, T., Laucou, V., Boursiquot, J.M., Javornik, B., & Bacilieri, R. (2015). Genetic clustering and parentage analysis of Western Balkan grapevines (*Vitis vinifera* L.) Vitis, *54* (Special Issue), 67-72.

Thomas, M.R., & Scott, N. (1993). Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). *Theoretical and Applied Genetics*, *86*, 985-990. https://doi.org/10.1007/BF00211051

Töpfer, R., Hausmann, L., & Eibach, R. (2011). Molecular breeding. In: Adam-Blondon A., Martinez-Zapater J., & C. Kole (Eds.), *Genetic, genomics and breeding of grapes*, 160-185. CRC Press.

*V*IVC (2007). *Vitis* International Variety Catalogue. http://www.vivc.de/index.php.

Žulj Mihaljević, M., Šimon, S., Pejić, I., Carka, F., Sevo, R., Kojić, A., Gaši, F., Tomić, L., Jovanović Cvetković, T., Maletić, E., Preiner, D., Božinović, Z., Savin, G., Cornea, V., Maraš, V., Tomić Mugoša, M., Botu, M., Popa, A., & Beleski, K. (2013). Molecular characterization of old local grapevine varieties from South East European countries. *Vitis*, *52*(2), 69-76.