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

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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 (IVC) was founded (Alleweldt, 1988). According to Maul et al. (2014), 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; Jakšič et al., 2013), having properties which allow them to be widely used - from variety identification to parent reconstruction and genome mapping (Seć 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; Seć et al., 1999; Adam-Blondon et al., 2004; Arroyo-Garcia and Martinez-Zapater, 2004; Di Gaspero et al., 2005; Merdinkolu et al., 2005; Goto-Yamamoto et al., 2006).
TABLE 1. Investigated grapevine varieties and their basic characteristics.

| Variety                                | Mean use | Colour of Skin | Colour of Flesh | Type of flower |
|----------------------------------------|----------|----------------|----------------|----------------|
| 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  |
| Kardarka kek                           | W        | N              |                | Hermaphrodite  |
| Koevidinka                             | W        | Rs             |                | Hermaphrodite  |
| Krajinski bojadiser                    | W        | N              | S              | Hermaphrodite  |
| Noir hätif 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 m².

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 MgCl₂ (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 1 min 30 s, 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 (Ho and He), the polymorphic information content (PIC) and the frequency of null alleles (Fnull) 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 consists of 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, .

| Locus   | No  | Ho   | He   | PIC   | Fnull | 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 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       | 1       | 7       | 3       | 2       | 1       | 1       | 4       | 1       |
| Babić veliki                                 | 5       | 3       | 3       | 3       | 1       | 1       | 1       | 1       | 3       | 4       | 3       | 1       | 5       | 3       | 3       | 4       |
| Braghina rosie                               | 5       | 3       | 7       | 3       | 3       | 1       | 1       | 1       | 4       | 7       | 3       | 3       | 3       | 5       | 5       | 1       |
| Bratkovina crna                              | 5       | 5       | 3       | 3       | 1       | 2       | 1       | 2       | 3       | 3       | 3       | 3       | 5       | 1       | 5       | 3       |
| Cabernet franc clon 21/20                    | 5       | 3       | 3       | 3       | 1       | 1       | 1       | 3       | 3       | 3       | 3       | 3       | 1       | 3       | 2       | 5       |
| Cabernet-Sauvignon                           | 5       | 7       | 5       | 1       | 1       | 1       | 1       | 1       | 3       | 3       | 4       | 4       | 3       | 3       | 1       | 3       |
| Cabernet-Sauvignon clon 10/32                | 5       | 7       | 5       | 1       | 1       | 1       | 1       | 3       | 3       | 4       | 4       | 3       | 3       | 1       | 1       | 3       |
| Cabernet-Sauvignon clon                      | 5       | 5       | 7       | 1       | 1       | 1       | 1       | 3       | 3       | 4       | 4       | 1       | 3       | 1       | 3       | 3       |
| Radmilovac                                   | 5       | 5       | 5       | 1       | 2       | 3       | 1       | 1       | 1       | 2       | 1       | 3       | 5       | 1       | 5       | 2       |
| Koevidinka                                   | 5       | 3       | 3       | 3       | 1       | 1       | 1       | 3       | 1       | 1       | 4       | 1       | 3       | 5       | 5       | 3       | 3       |
| Dinka mirisava                               | 5       | 3       | 3       | 1       | 1       | 1       | 1       | 1       | 1       | 3       | 1       | 2       | 7       | 2       | 5       | 3       |
| Blaufraenkisch                               | 5       | 3       | 3       | 1       | 1       | 2       | 1       | 1       | 1       | 3       | 1       | 2       | 7       | 2       | 5       | 3       |
| Gamay tenturier                              | 5       | 7       | 3       | 3       | 3       | 2       | 1       | 3       | 3       | 3       | 3       | 3       | 1       | 1       | 1       | 3       |
| Kadarun                                      | 5       | 3       | 5       | 5       | 3       | 1       | 1       | 2       | 5       | 3       | 3       | 3       | 7       | 5       | 3       | 3       |
| Krajinsko bojadiser                          | 5       | 7       | 3       | 3       | 3       | 2       | 1       | 3       | 3       | 3       | 3       | 3       | 1       | 1       | 5       | 3       |
| Lasina                                       | 5       | 3       | 3       | 3       | 2       | 1       | 1       | 2       | 3       | 3       | 3       | 2       | 1       | 2       | 1       | 3       |
| Cot                                          | 5       | 5       | 3       | 3       | 3       | 1       | 2       | 1       | 3       | 3       | 3       | 4       | 4       | 3       | 3       | 1       |
| Noir hâtif de Marseille                      | 5       | 5       | 3       | 1       | 1       | 2       | 1       | 1       | 1       | 3       | 3       | 3       | 2       | 7       | 3       | 3       |
| Piccola nera                                 | 5       | 3       | 3       | 3       | 3       | 1       | 2       | 1       | 2       | 3       | 3       | 3       | 1       | 1       | 5       | 3       |
| Pinot noir clon 658-12                       | 5       | 3       | 3       | 1       | 1       | 1       | 1       | 1       | 1       | 3       | 2       | 1       | 1       | 1       | 5       | 3       |
| Plavina mala                                 | 5       | 3       | 5       | 5       | 3       | 1       | 1       | 1       | 3       | 5       | 3       | 3       | 2       | 1       | 1       | 1       |
| Plavina velika                               | 5       | 3       | 7       | 7       | 3       | 1       | 1       | 2       | 7       | 4       | 4       | 3       | 3       | 5       | 5       | 3       |
| Pamid                                        | 5       | 3       | 3       | 3       | 1       | 1       | 1       | 3       | 3       | 3       | 3       | 3       | 5       | 5       | 5       | 4       |
| Prokupac                                     | 5       | 5       | 7       | 7       | 2       | 1       | 1       | 2       | 7       | 3       | 3       | 2       | 3       | 1       | 2       | 7       |
| Ruby Cabernet                                | 5       | 5       | 7       | 3       | 1       | 2       | 1       | 3       | 7       | 3       | 3       | 2       | 7       | 5       | 7       | 3       |
| Rudežuša crna                                | 5       | 3       | 3       | 3       | 1       | 1       | 1       | 3       | 3       | 3       | 1       | 1       | 5       | 5       | 5       | 3       |
| Kadarka kek                                  | 5       | 3       | 3       | 3       | 1       | 2       | 1       | 3       | 3       | 3       | 3       | 3       | 2       | 5       | 5       | 5       |
| Srpski rubin                                 | 5       | 5       | 3       | 3       | 1       | 2       | 1       | 1       | 3       | 3       | 3       | 3       | 1       | 9       | 2       | 3       |
| Stanušina crna                               | 5       | 3       | 3       | 3       | 1       | 1       | 1       | 1       | 3       | 2       | 2       | 2       | 2       | 1       | 2       | 5       |
| Vranac                                       | 5       | 3       | 3       | 3       | 1       | 2       | 1       | 2       | 3       | 3       | 3       | 2       | 1       | 1       | 1       | 3       |
| Župski bojadiser                              | 5       | 7       | 7       | 7       | 3       | 2       | 1       | 2       | 7       | 3       | 3       | 3       | 1       | 2       | 3       | 2       |
| Variety                  | OIV  | OIV  | OIV  | OIV  | OIV  | OIV  | OIV  | OIV  | OIV  | OIV  | OIV  | OIV  | OIV  |
|-------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Alicante Henri Bouschet | 1    | 1    | 1    | 5    | 5    | 3    | 3    | 9    | 5    | 5    | 5    | 2    | 3    |
| Babić veliki            | 1    | 1    | 1    | 1    | 3    | 3    | 3    | 9    | 5    | 3    | 3    | 2    | 3    |
| Braghina rosie          | 3    | 1    | 1    | 1    | 5    | 5    | 5    | 4    | 5    | 5    | 5    | 3    | 1    |
| Bratošina crna          | 1    | 1    | 1    | 1    | 3    | 3    | 9    | 4    | 9    | 5    | 5    | 5    | 2    |
| Cabernet franc clones 21/20 | 1    | 1    | 9    | 1    | 3    | 3    | 3    | 3    | 5    | 5    | 5    | 3    | 3    |
| Cabernet-Sauvignon      | 1    | 1    | 2    | 1    | 3    | 3    | 7    | 3    | 5    | 5    | 3    | 2    | 2    |
| Cabernet-Sauvignon clon 10/32 | 1    | 1    | 2    | 1    | 3    | 3    | 5    | 3    | 5    | 3    | 3    | 2    | 2    |
| Cabernet-Sauvignon clon Radmilovac | 1    | 1    | 2    | 1    | 3    | 5    | 3    | 5    | 5    | 3    | 3    | 2    | 2    |
| Koevidinka               | 1    | 1    | 1    | 9    | 5    | 5    | 3    | 3    | 9    | 5    | 3    | 3    | 2    |
| Dinka mirisava           | 1    | 9    | 1    | 1    | 3    | 5    | 3    | 3    | 9    | 5    | 3    | 1    | 1    |
| Blaufrankisch            | 3    | 1    | 1    | 1    | 3    | 3    | 3    | 5    | 3    | 5    | 3    | 1    | 3    |
| Gamay Tenturier          | 3    | 1    | 1    | 1    | 3    | 3    | 3    | 9    | 3    | 5    | 3    | 2    | 3    |
| Kadarun                  | 1    | 1    | 1    | 1    | 3    | 5    | 5    | 3    | 9    | 5    | 9    | 3    | 3    |
| Krajinski bojadiser      | 1    | 1    | 1    | 9    | 3    | 3    | 3    | 3    | 9    | 5    | 5    | 3    | 1    |
| Lasina                   | 1    | 1    | 1    | 1    | 1    | 3    | 5    | 3    | 9    | 5    | 3    | 3    | 1    |
| Cot                      | 3    | 1    | 1    | 1    | 3    | 3    | 5    | 3    | 9    | 5    | 3    | 3    | 1    |
| Noir hâtif de Marseille  | 1    | 1    | 1    | 1    | 1    | 3    | 3    | 3    | 5    | 5    | 5    | 3    | 1    |
| Piccola nera             | 1    | 1    | 1    | 1    | 1    | 3    | 5    | 3    | 9    | 3    | 5    | 3    | 1    |
| Pinot noir clon 658-12   | 1    | 1    | 1    | 1    | 5    | 1    | 3    | 3    | 9    | 3    | 7    | 3    | 1    |
| Plavina mala             | 1    | 1    | 1    | 1    | 5    | 3    | 5    | 3    | 9    | 5    | 5    | 3    | 3    |
| Plavina velika           | 1    | 1    | 1    | 1    | 5    | 3    | 5    | 3    | 9    | 5    | 5    | 3    | 3    |
| Pamid                    | 1    | 1    | 1    | 1    | 5    | 3    | 5    | 3    | 9    | 5    | 5    | 3    | 1    |
| Prokupac                 | 3    | 1    | 1    | 1    | 5    | 5    | 5    | 3    | 9    | 5    | 5    | 3    | 2    |
| Ruby Cabernet            | 1    | 1    | 1    | 1    | 5    | 1    | 3    | 3    | 7    | 7    | 5    | 5    | 2    |
| Rudežuša crna            | 1    | 1    | 1    | 1    | 3    | 3    | 1    | 3    | 9    | 5    | 5    | 3    | 1    |
| Kadarka Kek              | 1    | 1    | 1    | 1    | 5    | 3    | 3    | 3    | 5    | 5    | 3    | 1    | 3    |
| Srpski rubin              | 3    | 1    | 1    | 1    | 1    | 1    | 3    | 3    | 9    | 5    | 5    | 1    | 1    |
| Stanušina crna           | 3    | 1    | 1    | 1    | 3    | 3    | 3    | 3    | 9    | 5    | 7    | 3    | 2    |
| Vranac                   | 1    | 1    | 1    | 1    | 1    | 3    | 1    | 7    | 3    | 5    | 5    | 3    | 1    |
| Župski bojadiser          | 1    | 1    | 1    | 1    | 1    | 3    | 3    | 3    | 9    | 5    | 5    | 5    | 1    |

**TABLE 3. Ampelographic characteristics of investigated grapevine varieties (Part 2/3).**
## 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       | 3       | 5       | 5       | 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       |
| Kövedinka                      | 3       | 2       | 2       | 1       | 1       | 4       | 3       | 3       | 5       | 3       | 3       | 3       | 7       |
| Dinka mirisava                 | 5       | 2       | 3       | 1       | 1       | 1       | 3       | 5       | 5       | 3       | 3       | 3       | 5       |
| Blaufränkisch                   | 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       | 1       | 4       | 3       | 3       | 5       | 5       | 3       | 3       |
| Cot                             | 3       | 2       | 6       | 3       | 1       | 1       | 4       | 3       | 3       | 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       | 1       | 4       | 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       | 1       | 4       | 3       | 3       | 5       | 7       | 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       |
| Radežuša 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       | 1       | 4       | 3       | 3       | 5       | 5       | 3       | 3       |
| Stanušina crna                  | 3       | 4       | 6       | 1       | 1       | 1       | 4       | 3       | 3       | 5       | 3       | 3       | 7       |
| Vranac                          | 3       | 3       | 6       | 1       | 1       | 1       | 4       | 3       | 3       | 5       | 5       | 7       | 3       |
| 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 | VIC/VVMD27 | VIC/VVMD27 | 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 |
| Blaunfrankische | / | / | 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 | 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 | / | / | / | / |
| Radmilovac 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 Cot | 129 | 129 | 235 | 244 | 249 | 255 | 250 | 262 | 197 | 197 | 257 | 257 | / | / | 214 | 214 |
| Noir hâtif de Marseille | 129 | 137 | 233 | 266 | 255 | 279 | 258 | 272 | 205 | 207 | 257 | 267 | 254 | 266 | 205 | 220 |
| 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 | 256 | 264 | 197 | 201 | 259 | 273 | 286 | 286 | 211 | 218 |
| Rubi Cabernet | 129 | 131 | / | / | 255 | 255 | 256 | 258 | 194 | 201 | / | / | / | / | / | / |
| Radežuša crna | 129 | 139 | 227 | 233 | 255 | 269 | 264 | 272 | 197 | 197 | 257 | 273 | 264 | 266 | 205 | 218 |
| Kadarke Kek | 129 | 131 | 233 | 258 | 265 | 256 | 256 | 272 | 201 | 205 | 257 | 273 | 266 | 286 | 209 | 218 |
| Srpski rabin | 129 | 139 | 245 | 258 | 265 | 256 | 256 | 264 | 197 | 201 | 257 | 273 | 253 | 286 | 211 | 211 |
| Stanišinska 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 | 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 m².

In the second subgroup, the Plavina velika variety is more similar to the Kadarunk 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 IVVC 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://worldbestwines.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 IVVC 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 IVVC 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 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 IVVC 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, Koevdinkoka 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,
| 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    | 178 178 235 247 255 255 our data |
| Plavina-maločrn                               | 131  | 131   | 239    | 249    | 238    | 238    | 178 178 235 247 255 255 Štajner et al. (2014) |
| Maločrn                                       | 131  | 131   | 239    | 249    | 238    | 238    | 178 178 235 247 255 255 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    | 178 182 244 259 271 271 Lacombe et al. (2013) |
| 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 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 IVC database |
| BAGRINA_UREZANOGR_LISTA                      | 131  | 133   | 239    | 247    | 238    | 238    | 178 178 233 233 257 271 our data |
| Bagrina_SR                    | 131  | 133   | 239    | 247    | 238    | 238    | 176 178 233 233 257 271 Štajner et al. (2014) |
| LASINA                                      | 131  | 131   | 233    | 239    | 238    | 238    | 178 178 235 244 244 / our data |
| LASINA_CRO                                   | 131  | 131   | 233    | 239    | 238    | 238    | 176 178 235 245 245 255 IVC database |
| Lasina(#1642)                                | 131  | 131   | 233    | 239    | 238    | 238    | 176 178 235 245 239 255 Lacombe et al. (2013) |
| BABIČ_VELIKI                                 | 141  | 152   | 239    | 249    | 238    | 238    | 240 175 175 243 247 251 263 our data |
| Babic_CRO                                    | 141  | 149   | 247    | 249    | 238    | 238    | 176 176 243 247 239 251 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 IVC database |
| Bratkovaia,#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    | 178 244 244 271 271 our data |
| Plovdina_Crna_SRDB                           | 141  | 141   | 239    | 255    | 254    | 254    | 178 178 227 245 263 271 Štajner et al. (2014) |
| ALIKANT_BUŠE                                 | 141  | 141   | 243    | 257    | 240    | 254    | 178 178 235 247 251 251 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 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 m². 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 Plovdiva (Pamid). In IIVC (www.vive.de), only one variety was recorded under number IIVC 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 cvrana and Dinka mirisava was found to be in flower type: both varieties with the prefix ‘dinka’ have a hermaphroditic flower, while the Braghina rosie has a functionally female flower. In IIVC (www.vive.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 (Bešić 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. Bešić 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 Töpf er, 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 IIVC 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 Plovidnica 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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