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Genetic diversity of some autochthonous white grape varieties from Romanian germplasm collections

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Abstract: We investigated the phenotypic and genetic diversity among 25 grapevine accessions of white grape maintained in a germplasm collection *ex situ*. The selected varieties were characterised using standardised ampelographic descriptors and microsatellite markers to obtaintheir complete description. The ampelographic analysis based on 24 OIV descriptors and the obtained first dendrogram based on common features, revealed the extent of the phenotypic diversity ranging from 0.29 (between Coarnă albă and Chardonnay) to 0.92 (between Crâmpoşie and Cioinic), without any connection with their supposed origin or the current geographic area of distribution. The SSR allelic analysis with nine standard markers proved and confirmed its high discrimination potential for genotype identification and diversity. The second cluster analysis aimed to evaluate the genetic relationships among analysed accessions,based on Dice's similarity coefficient, revealed two groups of varieties: the first group (A) included twelve accessions with a degree of similarity between 0.37 and 0.5. The second (B) included eleven accessions, with a similarity degree between 0.33 and 0.67. The genotypic cluster analysis showed a distinct lower similarity between the Majarcă albă variety and all the varieties from the first group, and between Busuioacă de Bohotin and all the analysed varieties. The phenotypic and genetic matrices and dendrograms, although not correlated, were complimentary and provided valuable information about the diversity of the grapevine genotypes and the identification of the synonyms.

Keywords: genetic variability; molecular markers; phenotypic traits; similarity coefficient; *Vitis vinifera* L.

The cultivation of grapevines in the current territories of Romania seems to have begun during the seventh century BC (Constantinescu et al. 1970). The culture of grapevines on the territories of ancient Dacia and the continuation of this tradition throughout the history of its society is demonstrated by the numerous archaeological, epigraphic, and linguistic evidence. Nowadays, in addition to the international preferred varieties, the indigenous grapevine varieties, which are maintained and grown in small vineyards, have remained in the preferences of wine consumers (Antoce & Călugăru 2017). This was a strong reason

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for the Romanian grapevine research community to preserve and maintain as many varieties as possible. Between 1960 and 1970, germplasm collections for grapevines were established in nine research units that now maintain over 2600 accessions, of which, 30% are accessions considered old, local varieties. A complex and very detailed description of the accessions was made between 1959–1966 through a series of seven ampelographic books, later completed by Indreaş and Vişan (2001) and Rotaru (2009). Soon after, in addition to the ampelographic descriptors, molecular markers were added, with special attention paid to the complete description of the indigenous or economically important grapevine varieties (Bodea et al. 2009; Butiuc-Keul et al. 2010; Coste et al. 2010; Gheorghe et al. 2010; Gheţea et al. 2010, 2012; Popescu et al. 2017). The main condition for efficient management of germplasm collections is that all existing/introduced varieties in the collection must be correctly and completely described and identified. According to Upadhyaya et al. (2008), the characterisation of a germplasm means the registration of distinct, identifiable characteristics that are hereditary. The complete characterisation of the accessions involves: (a) the characterisation of morphological features following the International Organisation of Vine and Wine (OIV) Descriptor list for grape varieties and *Vitis* species' (OIV 2001), with 48 descriptors and the standardised methodology reported by Rustioni et al. (2014); (b) a genetic characterisation with at least nine SSR markers (VVS2, VVMD5, VVMD7, VVM25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG79), recommended by Maul et al. (2015). During the last 30 years, among the molecular markers, the microsatellite ones were the most widely used tool for identification of grapevine accessions, for the correct detection of synonyms, homonyms, or misnomers, and are considered the most efficient to highlight the degree of heterozygosity existing in a grapevine collection as well (Sancho-Galán et al. 2019; Bibi et al. 2020; Villano et al. 2022). Moreover, bringing concrete and striking proof, microsatellite markers overcome many of the limitations of phenotypic-based diversity analyses. Being a very important crop plant all over the world, the complete characterisation of accessions from national grapevine germplasm collections has been, and continues to be, an essential objective for the curators, farmers and consumers of grapevine derived products (Cipriani et al. 2008; Crespan 2010; Asaad et al. 2019; Sancho-Galán et al. 2019). Also, it is necessary to take care of any complex and unexpected changes caused by global warming that affect the grapevine plants. Also, the study of grapevine varieties of local/regional importance should be emphasised in light of global warming and site-specific adaptation (Naulleau et al. 2021). Possibly, some of the grape accessions, autochthonous and neglected ones could be useful resources to counteract the climate changes and ensure the wine-making production (Sancho-Galán et al. 2020). In Romania, out of a total of approximately 180 683 ha cultivated with vines for noble wine, including interspecific hybrids, the areas cultivated with varieties intended for the production of white wines exceeds 60%; white wines with a moderate alcohol content and being slightly aromatic are wine consumers' favourites. Starting from the above internationally accepted considerations and from the specifics of viticulture in Romania, we set out to present, in this paper, the morphological and genetic diversity of some accessions for white grape varieties, considered to be indigenous, existing in the germplasm collection belonging to the National Research and Development Institute for Biotechnology in Horticulture (NRDIBH) Stefanesti-Arges. Thus, the objectives of this study were: (1) to bring morphological and molecular evidence to light to demonstrate the genetic diversity of the 25 accessions; (2) to combine the morphological description with the molecular analysis to prove the grapevine accessions' identity and to identify possible synonyms, duplicated or misrecorded. The final results are the complete characterisation of some autochthonous accessions and the more efficient utilisation of the grape germplasm.

MATERIAL AND METHODS

Plant materials. For this study, 25 grapevine accessions for white grapes were selected (23 old varieties considered autochthonous and the 2 most internationally appreciated varieties, Chardonnay and Muscat Ottonel) grown in an *ex situ* collection belonging to the National Research and Development Institute for Biotechnology in Horticulture Ştefăneşti-Argeş (NRDIBH). The list of the analysed accessions with their registered number in the collection is presented in Table 1.

Morphological characterisation. The morphological aspects of the accessions were registered following the rules and criteria recommended as standard work for grapevine collections through the OIV descriptors (OIV 2001). From the list of the 48 total ampelographic descriptors, 24 descriptors were selected to be compared, for which distinct notations and differences among analysed accessions were registered. The descriptors were excluded from the calculations for which the same notation was registered. Thus, the following were considered: two descriptors for the young shoot (001 and 003), two descriptors for the young leaf (051 and 053), ten descriptors for the mature leaf (067, 068, 070, 076, 079, 080, 081-1, 081-2, 83-2 and 094), one descriptor for the sexual organs of the flower (151), three descriptors for the bunch characteristics (202, 204 and 208), three descriptors for the berry characteristics (223, 225 and 236) and three descriptors for the bunch technological characteristics (502, 503 and 505). All the data were recorded from the fifth year after plantation, at the proper moment of plant development, by a certain organ of the plant, and representing the correct description from at least five plants of the same grapevine accession.

Microsatellite analysis. From the obtained results with the plant material used to set up the *ex situ* germplasm collection, that was published previously (Popescu et al. 2017), the genetic profiles of the studied accessions with the following standard nine SSR markers were used: VVS2, VVM5, VVMD7, VVDM25, VVMD27, VVMD28, VVMD32, VrZAG79, VrZAG62. The efficiency of the nine primers to reveal the genetic diversity of the selected accessions was determined through the parameters calculated with the software CERVUS (Ver. 3.0.3.) (Kalinowski et al. 2007). The following measurements were considered with each of the nine SSR markers: the total number of alleles (Na); observed heterozygosity (Ho); expected heterozygosity (He); the polymorphic information content (PIC); the frequency of the null alleles (FNull) and the probability of identity (PI).

Clustering methods. To compare the two sets of data (morphological and molecular), for each one, the data were computed by NTSYSpc (Ver. 2.2.) (Rohlf 2009) and the distance matrix was obtained using the unweighted pair group method with arithmetic mean (UPGMA) algorithm based on the Dice coefficient (Dice 1945): one matrix and dendrogram for the morphological/phenotypic distance according to the 24 OIV descriptors and one cluster matrix and dendrogram according to the nine SSR markers' alleles frequency.

RESULTS AND DISCUSSION

Phenotypic variability based on the ampelographic description. All 25 accessions have, as the main features, short-time differences for the stage of ripening and very similar morphological features regarding the shape and size of the clusters. Comparing each accession to each other (Figure 1), a broad range of similarity coefficients for the 24 descriptors with distinct registrations was obtained (cluster matrix data not shown). The obtained dendrogram, as a result of the computed clustering method, is presented in Figure 2. The highest similarity coefficient for the ampelographic characteristics (0.92) was recorded between the (9) Crâmpoşie (cv. No. 9 in Figure 2) and Cioinic (6) varieties, both characterised by sterile pollen, the differences being for the shape of the blade

Table 1. Accessions with their registered code-number analysed in this study

No.	Analysed accessions	Registered No.	No.	Analysed accessions	Registered No.
1	Ardeleancă	ROM051-237	14	Frâncuşă	ROM051-254
2	Băşicată	ROM051-239	15	Galbenă de Odobeşti	ROM051-255
3	Berbecel	ROM051-240	16	Galbenă uriaşă	ROM051-256
4	Busuioacă de Bohotin	ROM051-038	17	Gordan	ROM051-257
5	Ceauş roz	ROM051-242	18	Gordin	ROM051-258
6	Cioinic	ROM051-243	19	Grasă de Cotnari	ROM051-108
7	Coada oilor	ROM051-244	20	Majarcă albă	ROM051-259
8	Coarnă albă	ROM051-246	21	Teişor	ROM051-270
9	Crâmpoşie	ROM051-241	22	Zemoasă	ROM051-273
10	Creaţă	ROM051-249	23	Zghihară de Huşi	ROM051-274
11	Cruciuliţă	ROM051-250	24	Chardonnay	ROM051-056
12	Fetească albă	ROM051-251	25	Muscat Ottonel	ROM051-138
13	Fetească regală	ROM051-253			

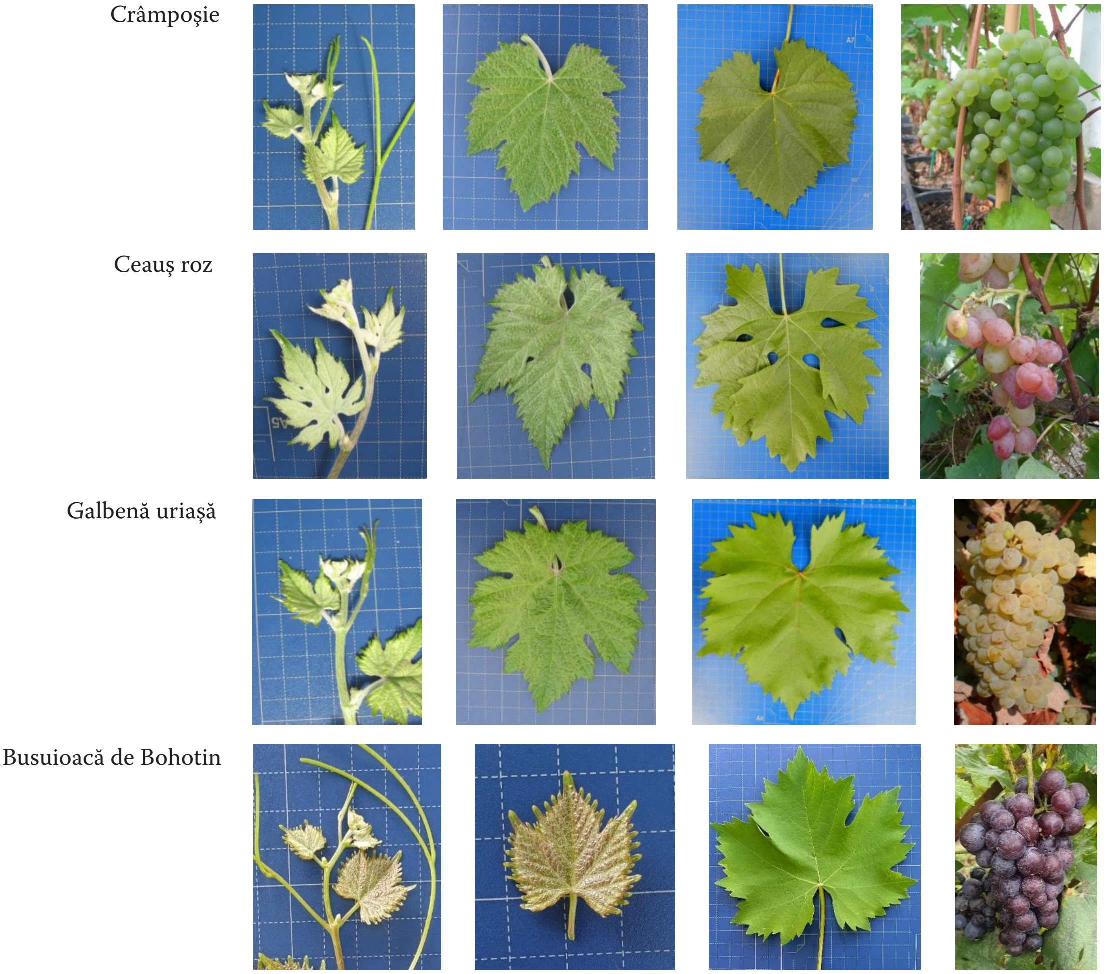

Figure 1. Variability in the grapevine accessions' ampelographic characteristics: shoot tip, young leaf, mature leaf, and bunch
The blue background for the shoot tip, young leaf, and mature leaf is with 1 cm^2 squares

and the colour of the main veins in the mature leaf. However, these two varieties have different origins: Crâmpoşie (9) comes from vineyards areas between the southern hills outside the Meridional Carpathians and the Danube; Cioinic (6) comes from vineyards located in the south-east of Romanian Moldavia (low-lying hills outside the Eastern Carpathians). A high value of the similarity coefficient (0.88) was calculated between Galbenă uriaşă (16) and Zghihară de Huşi (23). If Galbenă uriaşă (16) was grown here and there in ancient times in the eastern vineyards of Moldavia and Transylvania, it is present only in germplasm collections today, the variety Zghihară de Huşi (23) is now intensively cultivated in several vineyards from south-eastern Romanian Moldavia, being used for producing quality wines. Also, a high degree of similarity in terms of the morphological characteristics was recorded between the varieties Ardeleancă (1) and Băşicată (2) (0.83), although their growing area is different: Ardeleancă (1), as its name implies, has long been cultivated in the wine-regions of Transylvania, and Băşicată (2) was reported in the wine-growing regions of the north-eastern hills of Muntenia. The great resemblance between Fetească regală (13) and Chardonnay (24) (0.83) is due not only to the similar morphological characteristics, a special quality of the obtained wines, but also to their high potential for adaptation to different cultures' conditions. If Chardonnay (24) is one of the most widely grown varieties in the world, Fetească regală (13) (which belongs to the category of semi-aromatic varieties, just like Chardonnay) (24), is the second most cultivated in Romania (over 16 000 ha). It was discovered after 1920 in Transylvania, near Sighişoara and the molecular analyses confirmed its origin from Fetească albă (12) and Frâncuşă (14) (Laucou et al. 2018). A relatively high similarity coefficient of 0.75 was obtained in the case of two groups: Galbenă de

Odobeşti (15) with Gordan (17), and Berbecel (3) with Frâncuşă (14). In both groups, one accession differed from the others by six ampelographic descriptors. Regarding the Galbenă de Odobeşti (15) variety, it is known that it would originate from the vineyards of southeast Romanian Moldavia and is synonymous with Bătută neagră and Zghihară de Huşi (23) (Constantinescu 1958); Berbecel (3) has its origin from Oltenia (south-west of Romania), and Frâncuşă (14) is a high-yielding variety that is grown both in the vineyards of Moldavia and in Banat (southwestern Transylvania); the Gordan (17) variety was known in our country long before the phylloxera invasion and was mainly cultivated in vineyards in southern Romania and scattered ones in Transylvania. Teişor (21), or Frunză de tei, was most cultivated in Transylvanian vineyards and considered to be a synonym with Harslevelii (Constantinescu 1958). Zemoasă (22), an old, native variety, was known a long time before the invasion of phylloxera, was cultivated in vineyards from southern Moldavia. These two accessions showed a similarity of 0.67, the differences between them being mainly in terms of the quantity and quality of the grape juice. The same similarity was noticed between Busuioacă de Bohotin (4) and Muscat Ottonel (25). The Romanian variety Busuioacă de Bohotin (4) differs from Muscat Ottonel (25) by eight descriptors which refer to the characteristics of the young shoot, the degree of opening of the petiolar sinus, the presence of the tooth in the lateral sinuses and the colour of the berry skin. This variety has been cultivated since ancient times in the vineyards of Moldavia, it is distinguished for its productivity in different growing conditions, as well as for the special quality and particular flavour of the berry. The Ceauş roz (5) variety was widely grown in Dobrogea and in some vineyards in Transylvania, and the (18) Gordin variety was known in the vineyards of southern Romania. These two varieties are now present only in germplasm collections and differ from one another by nine characteristics, among which are: the density of the prostrate hairs on the lower side of the young leaves, the number of lobes, the shape of the teeth and the depth of the upper lateral sinuses of the mature leaves, the density of the bunches and the skin colour of the berries. The lowest similarity coefficient of 0.29 was obtained by comparing the morphological descriptors between Coarnă albă (8) and Chardonnay (24). Among the analysed accessions, Coarnă albă (8) is the only one that produces grapes for fresh consumption, with good resistance to storage in winter, has a leaf with

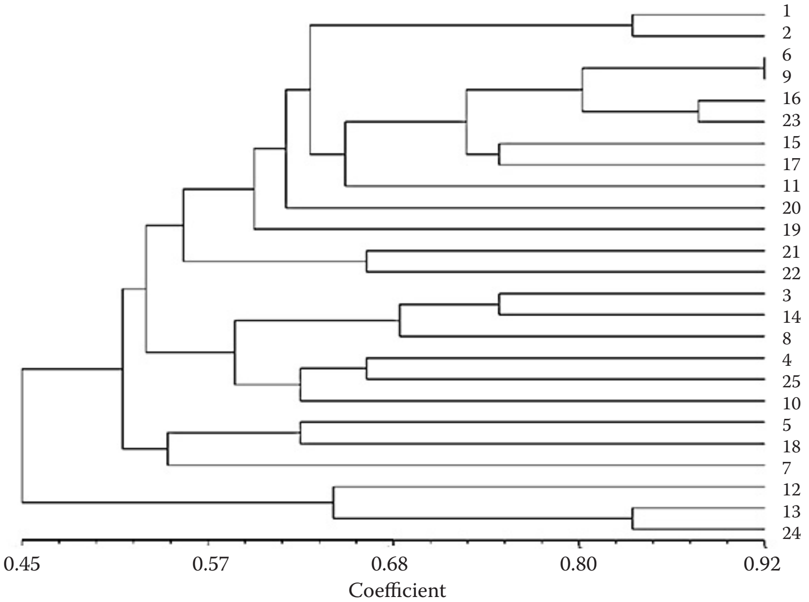

Figure 2. The unweighted pair group method with an arithmetic mean (UPGMA) dendrogram based on the standardised OIV descriptors for the 25 grapevine accessions

1 – Ardeleancă; 2 – Băşicată; 3 – Berbecel; 4 – Busuioacă de Bohotin; 5 – Ceauş roz; 6 – Cioinic; 7 – Coada oilor; 8 – Coarnă albă; 9 – Crâmpoşie; 10 – Creață; 11 – Cruciuliță; 12 – Fetească albă; 13 – Fetească regală; 14 – Frâncuşă; 15 – Galbenă de Odobeşti; 16 – Galbenă uriaşă; 17 – Gordan; 18 – Gordin; 19 – Grasă de Cotnari; 20 – Majarcă albă; 21 – Teişor; 22 – Zemoasă; 23 – Zghihară de Huşi; 24 – Chardonnay; 25 – Muscat Ottonel

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an overlapping petiolar sinus and the flowers are with recurved stamens, functionally female, and produce sterile pollen. Regarding its origin, it is supposed that Coarnă albă (8) was brought from Turkey many centuries ago and it acclimatised so well in Romania that many specialists consider it a local variety (Constantinescu et al. 1959). This trait of functionally female flowers, characterised either by well-developed ovaries, and anthers with filaments reflexed outwards (specific for Cârcioasă, and Coarnă albă (8)), or by hermaphrodite flowers with anthers inclined outwards (specific for Crâmpoşie, Ceauş roz and Cioinic), is considered as an ancestral characteristic, related to *Vitis vinifera* domestication (Fechter et al. 2012). Female flower cultivars are common among *proles orientalis* (Cattonaro et al. 2014), but are less grown in western European vineyards due to pollen sterility and berry set issues. The evolution of the flower type within the genus *Vitis* is an exciting subject of study. Although the main genes involved in determining the type of flower are known (Coito et al. 2017), the evolution over time from the male and female flower types specific for sylvestris to the hermaphrodite flower type specific for most vinifera varieties (Riaz et al. 2013), as well as the complex genetic interaction correlated to the sexual differences in grapevine (Ramos 2021) are still under genomic studies. In Romania, the preservation of this type of variety in vineyards and in germplasm collections was due either to obtaining a varietal diversity for its very good qualities as both a table and wine grape or to use it in the cross-hybridisation process as female parents (Dejeu 1995; Popescu & Teodorescu 2004). Chardonnay (24) is a well-known and appreciated grapevine variety all over the world used for the production of superior dry, semidry, or sweet wines depending on the pedoclimatic conditions in which it is grown. Although the 'Chardonnay' variety is widespread in almost all European wine-growing countries, its provenance is still unknown (Bowers et al. 1999; Vouillamoz et al. 2004).

Genetic diversity based on SSR markers. The parameters widely used to show the genetic diversity among the 25 analysed accessions were obtained and are presented in Table 2. A total number of 71 alleles were recorded with the nine tested SSRs, ranging between five (VVMD25) and eleven (VVMD28) alleles per locus, and an average of 7.88 alleles per locus was registered. Aiming to obtain the quantification of the extent of the genetic variability among the analysed accessions, the parameters for heterozygosity were considered. The values obtained for the observed heterozygosity (Ho) varied between 0.56 (VVS2) and 1.0 (VVMD5), with a mean value of 0.866; the expected heterozygosity (He) varied between 0.692 (VVMD7) and 0.871 (VVMD28), with a mean value of 0.79. Similar situations with higher Ho values in comparison to He can be found in numerous reports (Ibañez et al. 2003; Martinez et al. 2006; Carimi et al. 2010; Boz et al. 2011). The obtained quantum of these two parameters proves the high variability or the gene diversity among the analysed accessions. As was expected, the highest number of alleles per locus and the highest obtained He value with the VVMD28 marker, were directly correlated with the highest PIC value of 0.837. An av-

Table 2. Genetic diversity parameters of the nine SSRs with the 25 analysed accessions

SSR marker	Na	Ho	He	PIC	F(Null)	PI
VVS2	8	0.56	0.736	0.680	0.1325	0.119
VVMD5	8	1.00	0.852	0.815	–0.0943	0.047
VVMD7	7	0.76	0.692	0.633	–0.0579	0.149
VVMD25	5	0.96	0.756	0.695	–0.1321	0.113
VVMD27	8	0.84	0.808	0.762	–0.0292	0.073
VVMD28	11	0.92	0.871	0.837	–0.0384	0.038
VVMD32	8	0.92	0.798	0.752	–0.0831	0.077
VrZAG62	7	0.92	0.781	0.726	–0.0938	0.094
VrZAG79	9	0.92	0.816	0.775	–0.0784	0.065
Average ± SD	7.88 ± 1.61	0.866 ± 0.13	0.79 ± 0.056	0.741 ± 0.065	–0.052 ± 0.07	0.086 ± 0.036 1.22×10^{-14}

Na – the total number of alleles; Ho – observed heterozygosity; He – expected heterozygosity; PIC – polymorphic information content; F(Null) – estimated frequency of null alleles; PI –probability of identity; SD – standard devaition erage PIC value of 0.741 proved the efficiency of the nine SSR markers to evaluate the genetic diversity among the analysed accessions. The results with the Romanian accessions are very similar to those reported by Lemos Serrote et al. (2020), Augusto et al. (2021), Milišić et al. (2021) and showed the discriminatory power of the tested molecular markers, being classified as highly informative. The null allele frequencies represent a parameter of which value is in inverse proportion to the diversity. The values expressed mathematically with the Cervus program, are adequate for large populations and represent the frequency of non-functional alleles (as results of mutations) characterised by a high frequency of mutations of the binding sites for microsatellite primers (Dakin & Avise 2004; Kalinowski et al. 2007). This feature is important because genetic distances based on microsatellites are usually considered for the construction of dendrograms of the possible related genotypes, and should not influence the aspect of the final tree topology (Wen et al. 2013). In the case of our experiment, although the microsatellite null alleles were evaluated in a relatively small number of accessions, their values had significance in revealing the genetic diversity among the analysed genotypes and are clearly correlated with the other analysed parameters. Thus, we found that VVS2 had the highest estimated probability of a null allele (0.132), being in correlation with the lowest value of the Ho, while VVMD5 and VVMD25 registered significantly low null alleles probability (–0.0943 and –0.1321, respectively) in opposition to the highest values for Ho (1.00 and 0.96, respectively). Analysing the probability of the identity (PI) for all the loci, our results showed that VVMD7 and VVS2 could be considered the least discriminating loci with the highest PI value and the lowest PIC value, in opposition to VVMD28 and VVMD5, considered as the most discriminating loci having the lowest PI and the highest PIC values. In total, the cumulative probability of the identity for all the analysed accessions was 1.2×10^{-14}. Similar to Hvarleva et al. (2005), and Sefc et al. (2000), our data showed that a higher PI value, as a measure of the probability to have identical genotypes in a population, is directly correlated either with the low number of alleles or with the lowest PIC values.

Genetic relatedness. The matrix of the similarity coefficients (data not shown) as measurements of the similarities between all the pairs of analysed accessions represents a mathematic evaluation of the degree of genetic variability among the analysed varieties. Based on the allele frequencies for the nine SSRs, the obtained values ranged between 0.06 and 1. The lowest similarity values, as a result of the identification of a single common allele, were obtained in the case of the following variety combinations: Berbecel with Busuioacă de Bohotin, Berbecel with Frâncușă, Coarnă albă with Fetească albă, Fetească albă with Cioinic, Chardonnay with Fetească albă and Chardonnay with Fetească regală. This small value (0.06) can either be proof that there is no genetic link between the varieties enclosed in the same group (Berbecel and Busuioacă de Bohotin) or that the two varieties belong to different clusters. As expected, the genetic identity between the Gordan and Zemoasă accessions was confirmed, as well as between Galbenă de Odobești and Zghihară de Huși (Popescu et al. 2017). These four varieties are considered Romanian varieties, which were cultivated long before the invasion of phylloxera. The Gordan variety has been cultivated since ancient times in the vineyards of Oltenia (southwestern Romania), while Zemoasă was grown scattered through vineyards from northeast of Romanian Moldavia, and are both now present only in germplasm collections. The other two varieties, Galbenă de Odobești and Zghihară de Huși are very well known as local, old varieties that were grown in vineyards from the south of the Romanian Moldavia wine regions and were used to obtain wines each under its own name, as a different variety Galbenă de Odobești and Zghihară de Huși, respectively. From a morphological point of view, the resemblance between these two groups of varieties was recorded in old documents (Constantinescu 1958). Although they were considered to belong to the same group (Constantinescu et al. 1960), they were separately described, grown in different vineyards, and used to obtain a certain type of wine. Our ampelographic description showed a degree of similarity of 0.67, for Gordan and Zemoasă, and 0.79 for Galbenă de Odobești and Zghihară de Huși, that could be explained by the long-time culture in different agro-climatic conditions and areas (Constantinescu et al. 1960). The molecular analyses proved and confirmed that the accessions from the mentioned two groups are identical from a genetic point of view. The Dice coefficients from the matrix, as the parameters for genetic similarity among analysed accessions, were used to obtain the dendrogram presented in Figure 3. This dendrogram indicates the separation of the accessions into two

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main groups which is clearly different from the dendrogram obtained for the morphological descriptors. The first cluster (A) groups together twelve accessions which, according to old documents, have their origin in different wine-growing areas of Romania. Of these, five accessions (Fetească regală, Fetească albă, Crâmpoșie, Grasă de Cotnari and Frâncușă) are grown in large production vineyards, while the rest are only present in germplasm collections. The data confirmed previously published works (Lacombe et al. 2013; Popescu et al. 2017) that proved a possible parent-offspring among Fetească regală that could be the progeny of Fetească albă and Frâncușe. With the exception of the Gordan and Zemoasă accessions, which proved to be synonyms in this group, the degree of similarity was between 0.5 (Crâmpoșie with Fetească regală) and 0.61 (Ardeleancă with Bășicată, and Frâncușă with Teișor). For the first cluster of accessions, the Majarcă albă variety is distinguished. It seems to have originated in the territories of the former Yugoslavia, where it has long been known as Slankamenka bela (Constantinescu et al. 1960). In Romania, this variety was first cultivated in Banat (wine-growing regions in western Romania), later in Transylvanian vineyards, and is considered a variety well adapted for sand cultivation. As we expected, and also according to the previous results (Lacombe et al. 2013), Majarcă albă has the same SSR profile as Slankamenka bela. Over time, Majarcă albă has been integrated into the vineyard cultural area. According to our results, the clustering analysis by the unweighted pair group, this accession has a low degree of similarity with all the other varieties in this cluster, between 0.17 (with Fetească albă) and 0.39 (with Grasă de Cotnari). The second cluster (B), which consists of eleven accessions, includes: the two internationally recognised varieties (Chardonnay and Muscat Ottonel), a table grape variety (Coarnă albă), two varieties that have proven to be synonyms (Galbenă de Odobești and Zghihară de Huși) and six other varieties that are now only present in germplasm collections and in scattered private vineyards. The obtained dendrogram shows, for this cluster, a degree of similarity between 0.39 (Chardonnay with Muscat Ottonel) and 0.67 (Coarnă albă with Creață). From all the accessions, the Busuioacă de Bohotin variety is distinguished. Although its origin is still uncertain, in Romania, it is considered one of the most appreciated autochthonous varieties, originally from Moldavia – the village of Bohotin, in the Iași wine-growing region. Due to its good yield potential in different environmental conditions, the

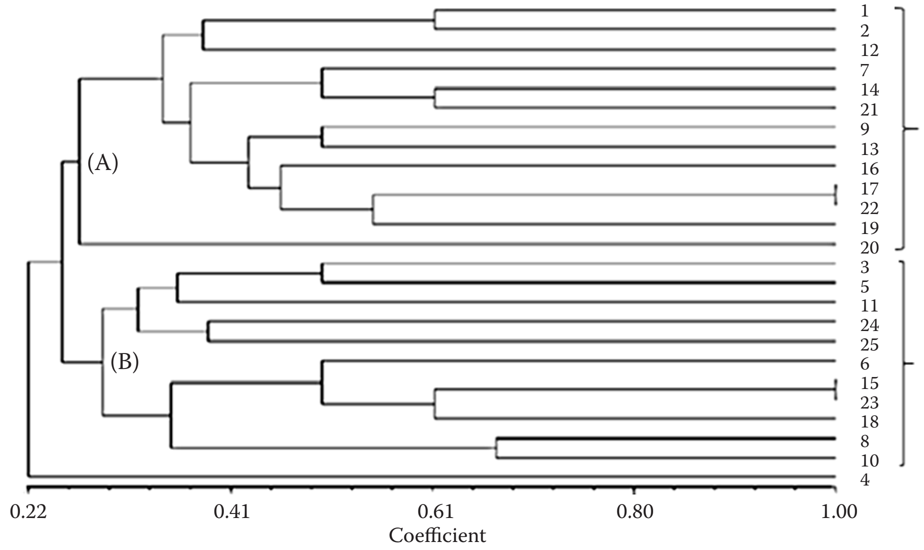

Figure 3. The unweighted pair group method with an arithmetic mean (UPGMA) dendrogram based on the microsatellite loci data for the 25 grapevine accessions

1 – Ardeleancă; 2 – Bășicată; 3 – Berbecel; 4 – Busuioacă de Bohotin; 5 – Ceauș roz; 6 – Cioinic; 7 – Coada oilor; 8 – Coarnă albă; 9 – Crâmpoșie; 10 – Creață; 11 – Cruciuliță; 12 – Fetească albă; 13 – Fetească regală; 14 – Frâncușă; 15 – Galbenă de Odobești; 16 – Galbenă uriașă; 17 – Gordan; 18 – Gordin; 19 – Grasă de Cotnari; 20 – Majarcă albă; 21 – Teișor; 22 – Zemoasă; 23 – Zghihară de Huși; 24 – Chardonnay; 25 – Muscat Ottonel

accumulation of significant amounts of sugars in the grapes, and good potential for production of aromatic wines with specific colours, the cultivation area has increased. With this accession, through clustering analysis by the UPGMA method, genetic similarity coefficients between 0.06 (with Berbecel) and 0.33 (with Coarnă albă and also with Grasă de Cotnari) were obtained.

General considerations. Knowing the origin of the varieties and their area of distribution are useful for understanding the history of the grapevine domestication and the evolution of its growing surface. Until 50 years ago, only the old documents presenting information and opinions of different ampelographers were considered, together with their morphological descriptions of grapevine varieties. Sometimes, this information was empirical, incomplete, and subsequently proved to be insufficient until the standardisation of the system of notation and registration of the characteristics. For the present investigation, we applied internationally recognised procedures for the characterisation of the 25 accessions. All the ampelographic features were used to obtain the dendrogram after the calculation of the similarity coefficients. This dendrogram represents the image of the mathematical proportion of the common morphological features, without any connection with the spread of the growing area of the accessions, or with their geographic origin as known from old documents. Morphological differences among accessions could be useful to prove the importance of the cultural area for the expression of the yield quality and also for the potential of adaptation of each variety to different edaphic and climatic conditions. If local conditions and similar applied technologies have determined the stability of certain morphological traits, the expression of the phenotypic plasticity is essential for adaptation to environmental conditions in permanent change. Besides the morphological characterisation, the molecular analysis with the set of standard SSR markers represents the main condition for obtaining a complete description, thus ensuring the accuracy of the discrimination of the grapevine varieties and with the comparison of the results in international databases (Ferlito et al. 2018; Karataş 2019; Sargolzaei et al. 2021; Cretazzo et al. 2022). In addition, it is possible to identify synonymous, homonymous, or incorrectly registered varieties. Germplasm collections are invaluable sources of autochthonous genetic resources that can be harnessed in breeding programmes, along with the preferred genotypes on the international market. The establishment of germplasm collections and the preservation of their richness require the use of both types of markers (morphological and molecular) for the correct identification of each accession and the efficient use of the preserved diversity. As with many other authors (Martinez et al. 2003; Stavrakaki & Biniari 2017; Margaryan et al. 2021), our results demonstrated the effectiveness of the ampelographic and molecular methods for assessing the genetic diversity among the grapevine accessions. Each type of marker, analysed by appropriate mathematical methods, brings additional and complementary information, which contributes to the correct assessment of the intra- and inter-varietal diversity of the grapevine genetic resources.

CONCLUSION

Standardised protocols for ampelographic description and molecular characterisation were used to obtain the complete and comprehensive identification of 23 indigenous grapevine accessions for white grapes in parallel with two of the most international appreciated varieties (Chardonnay and Muscat Ottonel). The morphological description, their similarity coefficients, and the obtained UPGMA dendrogram showed the high variability in the ampelographic characteristics among the analysed accessions, and also the degree of resemblance among the pairwise groups of the grapevine genotypes ranging between 0.29 (Chardonnay with Coarnă albă) and 0.92 (Crâmpoșie with Cioinic). Molecular analysis with the nine standard SSR markers allowed us to confirm the identification of the autochthonous accessions either as synonymous (Gordan with Zemoasă, and Galbenă de Odobești with Zghihară de Huși) or as distinct genotypes. The parameters used as genetic diversity indicators provided certainty for the efficiency of the SSR markers in the genotype discrimination. The individual estimated values representing the quantification of the genetic diversity among the analysed accessions proved each microsatellite's efficiency, and also indicated their power to distinguish each genotype one from another. Both types of analysed data (ampelographic and molecular), without any correlation between them, were efficient to reveal the diversity of the plant material from our germplasm collection and were considered complementary for the complete description of the accessions.

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