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ASSESSMENT OF GENETIC DIVERSITY OF GRAPEVINE GENOTYPES USING SIMPLE SEQUENCE REPEATS (SSR) MARKERS AND SSR-BASED DNA BARCODE DESIGN

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Abstract

Romania has a valuable wine heritage, and the genetic evaluation of grapevine genotypes from Vitis germplasm collections is a national priority. Thus, this study aimed to characterize 22 Romanian grapevine genotypes and five international reference varieties using nine SSR (Simple Sequence Repeats) loci. VVMD32 was the most informative (He=0.837; PI= 0.040) SSR marker with 11 alleles generated. Conversely, the VvZag62 locus generated the lowest number of alleles (6) with values recorded for He=0.744 and PI= 0.109. The genetic SSR profiles were used to construct a barcode design to compare the number and size of alleles among the screened grapevine samples. The results of this study show that SSR genotyping supplemented with a useful data grouping mode is effective for genetic diversity analysis of Vitis germplasm resources.

Key words*: Vitis vinifera L., OIV microsatellites, DNA polymorphism, genetic resources.*

INTRODUCTION

Romania is a Southeastern European country with a long grapevine cultivation and winemaking tradition (Bodea et al., 2009). Over the centuries, the winemaking areas have been developed into favourable geographical areas for viticulture in Romania, depending on the relief, climatic conditions, and soil types (Soare et al., 2010; Bănuță et al., 2024).

Grapevine cultivation has existed since ancient time, and Romanian grape germplasm collections showcase various gene pools. These include autochthonous varieties, old local biotypes, and newly developed cultivars that have arisen from utilizing the genetic resources of both native and international varieties, interspecific hybrids, and wild grapevine populations of *Vitis vinifera* ssp. *sylvestris* (Popescu et al., 2018). Additionally, germplasm mixing occurred due to the import of different genotypes following the phylloxera epidemic (Pușcă, 2010). Ampelographic characterization and SSR molecular marker analysis of grapevine genotypes have been performed across various national germplasm collections in several European countries (Agar et al., 2011; Buhner-Zaharieva et al., 2010; Dzhambazova et al., 2009; Grando & Frisinghelli, 1998; Lefort & Roubelakis-Angelakis, 2000; Lopes et al., 1999; Martin et al., 2003; Pellerone et al., 2001; Štajner et al., 2011). These studies aimed to compile the results in the national *Vitis* databases or to coordinate efforts among them through the European *Vitis* database (Maul et al., 2012).The molecular evaluation of grapevine genotypes maintained in Romanian germplasm collections using SSR markers is still a work in progress. Some previously published studies (Gheorghe et al., 2010; Motoc et al., 2010) suggest that SSR markers are efficient and reliable tools for accurately characterising Romanian grapevine varieties. They complete the classical methods of identification based on

ampelographic description (Popescu & Crespan, 2018).
Identifying SSR genetic profiles for old local varieties and newly developed cultivars can serve as a passport certifying their authenticity. This approach also represents a critical strategy for the future preservation of Romanian grapevine accessions with scientific and economic significance (Ghețea et al., 2012; Ilina-Dumitru et al., 2024).
The current study utilized nine nuclear SSR loci to characterize 22 Romanian grapevine varieties, with five international cultivars included as comparative genetic profiles. The microsatellite analysis was employed to create a barcode system, a visual representation of the data that simplifies the detection of genotypic differences.

MATERIALS AND METHODS

Total genomic DNA isolation and PCR amplifications were carried out according to the protocol presented by Hârța & Pamfil (2013).
Table 1 presents the *Vitis vinifera* genotypes used for SSR genetic analysis.

Table1. Plant material used for SSR analysis

Genotype name	Berry colour	Geographic origin	Distribution code*
Alb aromat	white	Timiș county	330
Alb crocant	white	Timiș county	330
Alb lax	white	Timiș county	330
Arămiu	white	Timiș county	330
Auriu	white	Timiș county	330
Coada oii	white	Timiș county	330
Conic auriu	white	Timiș county	330
Gras aripat	white	Timiș county	330
Ochiul boului	white	Timiș county	330
Pătrunjarca	white	Timiș county	330
Răschirata	white	Timiș county	330
Cioinic	white	Iași county	320
Coarna albă	white	Iași county	320
Creața de Banat	white	Iași county	320
Cruciuliță	white	Iași county	320
Feteasca albă	white	Iași county	320
Feteasca regală	white	Iași county	320
Frâncușă	white	Iași county	320
Galbenă de Odobești	white	Iași county	320
Grasă de Cotnari	white	Iași county	320
Mustoasa de Măderat	white	Iași county	320
Zghihară de Huși	white	Iași county	320
Cabernet Sauvignon-RG**	white	Iași county	310
Muscat Ottonel-RG**	white	Iași county	310
Riesling italian-RG**	white	Iași county	310
Chardonay blanc-RG**	white	Iași county	310
Gewuerztraminer-RG**	rose	Iași county	310

In Table 1, the distribution codes were assigned according to Popescu & Crespan (2017): *310 = local cultivar, spread all over, international cultivar; 320 = major local cultivar, of local importance, but widely grown and 330 = minor local cultivar, of local importance, fairly grown; **RG denotes the reference genotypes.
Nine SSR loci were employed for SSR analysis: VVS2 (Thomas & Scott, 1993); MD5, MD7 (Bowers et al., 1996); MD25, MD27, MD28, MD32 (Bowers et al., 1999); VrZAG 62, VrZAG 79 (Sefc et al., 1999). These SSR markers are recognized as OIV (International Organization of Vine and Wine) descriptors (https://www.oiv.int/public/medias/6886/oiv-viti-609-2019-en.pdf).The features of the SSR primers used for analysing *Vitis vinifera* genotypes are displayed in Table 2.

Table2. Primer name and sequences used for SSR analysis

SSR primer name	Primer sequences (5`-3`)	OIV code
VVS2 F	CAGCCCGTAAATGTATCCATC	801
VVS2 R	AAATTCAAAATTCTAATTCAACTGG	801
VVMD5 F	CTAGAGCTACGCCAATCCAA	802
VVMD5 R	TATACCAAAAATCATATTCCTAAA	802
VVMD7 F	AGAGTTGCGGAGAACAGGAT	803
VVMD7 R	CGAACCTTCACACGCTTGAT	803
VVMD 27 F	GTACCAGATCTGAATACATCCGTAAGT	804
VVMD 27 R	ACGGGTATAGAGCAAACGGTGT	804
VrZAG 62 F	GGTGAAATGGGCACCGAACACACGC	805
VrZAG 62 R	CCATGTCTCTCCTCAGCTTCTCAGC	805
VrZAG 79 F	AGATTGTGGAGGAGGGAACAAACCG	806
VrZAG 79 R	TGCCCCCATTTTCAAACTCCCTTCC	806
VVMD 32 F	TATGATTTTTTAGGGGGGTGAGG	807
VVMD 32 R	GGAAAGATGGGATGACTCGC	807
VVMD 25 F	TTCCGTTAAAGCAAAAGAAAAAGG	808
VVMD 25 R	TTGGATTTGAAATTTATTGAGGGG	808
VVMD 28 F	AACAATTCAATGAAAAGAGAGAGAGAGA	809
VVMD 28 R	TCATCAATTTCGTATCTCTATTTGCTG	809

The sizes of alleles/ locus were evaluated using the CEQ fragment analysis software. Genetic parameters, including allele frequencies, expected and observed heterozygosity, estimated null allele frequencies, and the probability of identity, were calculated using Identity 1.0 software (Wagner & Sefc, 1999). DNA barcodes were organized in Microsoft Excel according to the protocol described by Galbacs et al. (2009).

RESULTS AND DISCUSSIONS

Nine SSR primer pairs amplified 77 SSR alleles, with an average of 8.55 alleles per locus, comparable to results obtained in previous reports (Coste et al., 2010; Carimi et al., 2010). The highest number of alleles was for VVMD32 locus (11), while the lowest for VrZAG62 locus (6). To evaluate the efficiency of microsatellite markers, we examined observed (Ho) and expected heterozygosity (He) to assess the

genetic variability among the analysed grapevines. Ho varied from 0.744 for VrZag62 to 0.868 for VVMD28. VVMD28 was the most informative SSR marker (He=0.868; PI= 0.031). Conversely, VvZag62 locus generated the lowest number of alleles (6) with recorded values for He=0.744 and PI= 0.109. The mean of observed heterozygosity (Ho) was slightly higher (0.826) than the expected value (0.802), with significant differences observed locus by locus (Table 3).

Table 3. Genetic parameters highlighting the genetic variability among the 27 grapevine genotypes

Locus	Allele size range (bp)	No. of alleles	He[1]	Ho[2]	Estimated frequency of null alleles	PI[3]
VVS2	133-153	8	0.778	0.629	+ 0.083	0.078
VVMD5	228-248	9	0.831	0.814	+ 0.008	0.048
VVMD7	235-257	8	0.773	0.740	+ 0.018	0.083
VVMD25	239-269	8	0.770	0.888	- 0.066	0.087
VVMD27	176-195	8	0.796	0.851	- 0.030	0.071
VVMD28	218-268	10	0.868	0.962	- 0.050	0.031
VVMD32	240-273	11	0.836	0.814	+ 0.011	0.040
VrZAG62	186-204	6	0.744	0.851	- 0.061	0.109
VrZAG79	237-259	9	0.831	0.888	- 0.031	0.044
Mean		8.55	0.802	0.826		

[1]Heterozygosity expected (He);
[2]Heterozygosity observed (Ho);
[3]Probability of identity (PI).

The mean observed heterozygosity (82.6%) was higher than that reported for other grapevine germplasm collections, such as Greece, with 73.7% (Bibi et al., 2020), and Turkey with 73.1% (Yüksel et al., 2023). It was also comparable to the values obtained by Lacombe et al. (2013) and Popescu & Crespan (2018). The alleles of the markers used in this study were evenly distributed among analysed genotypes, resulting in a low probability of identical genotypes (0.031-0.087). This was the case except for the VrZAG79 marker, which had a PI value of 0.109 as shown in Table 3. DNA microsatellite analysis was used to create a barcode design. This study's findings demonstrated that this data grouping system was effective in characterizing Romanian grapevine genotypes. The SSR barcode system visualizes data and facilitates the detection of genotypic differences. If an overlap occurs in the allele size representation (where two or more markers have the same allele sizes), the bar can be marked with an index indicating those differences (Galbacs et al., 2015; Chinnappareddy et al., 2012). In this study, the SSR results were converted into DNA barcodes by separating the allele sizes from each SSR locus and then sorting the allele size data from lowest to highest.

As shown in Figure 1, the allele size bars were created on a linear scale for all the analysed cultivars. This method of data grouping can be beneficial for further studies regarding the characterization of Romanian grapevine cultivars at the molecular level. Incorporating these DNA barcodes into a future Romanian-designed *Vitis* database could enhance the quality of genetic resource management in grapevines.

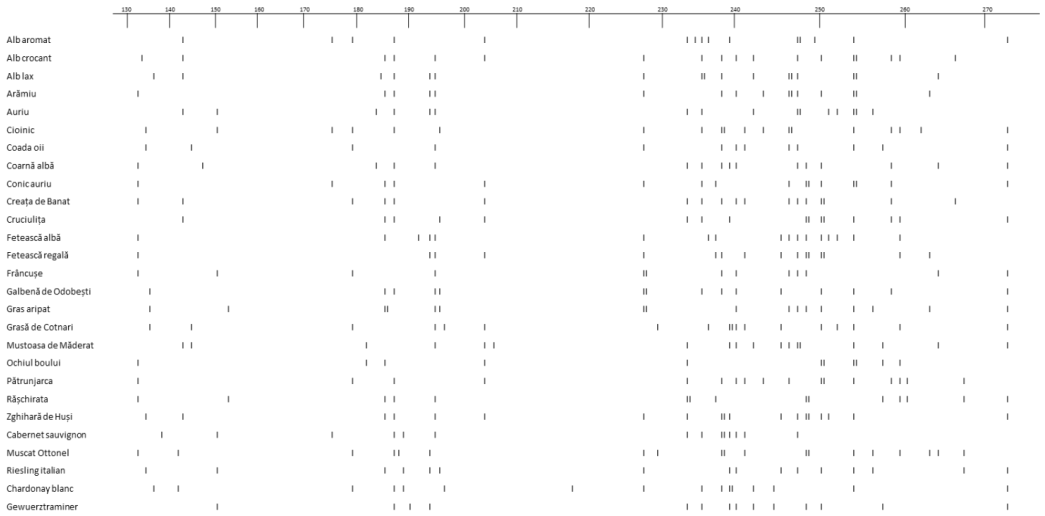

Figure1. SSR-based DNA barcodes of the studied grapevine genotypes

CONCLUSIONS

Although Romania has a valuable viticultural heritage, efforts to characterize grape varieties at the DNA level are still less emphasized compared to research results in other countries regarding molecular ampelography. In this study, the SSR genetic profiles of eleven *Vitis vinifera* genotypes (local biotypes) from Banat were identified for the first time, highlighting the genetic relationships with reference varieties and other Romanian varieties.

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