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Genetic diversity of some Romanian grapevine cultivars as revealed by microsatellite markers

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RALUCA NICOLETA GHEORGHE[1], CARMEN FLORENTINA POPESCU[1], DORU PAMFIL[2], CRISTINA NICOLETA CIOCIRLAN[1], RADU SESTRAS[2]
[1]National Research and Development Institute for Biotechnology in Horticulture, Sos. Bucuresti-Pitesti, no. 37, 117715, Stefanesti, Arges, Romania, gheorghe_raluca2004@yahoo.com
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Manastur Street, Cluj-Napoca, Romania

Abstract

The PCR-based DNA microsatellite analysis has been applied to define genetic relationships among eleven Romanian grapevine varieties. 87 alleles with a mean number of 7.90 alleles per locus were detected in the set of cultivars analyzed. The total number of alleles per locus varied between 4 (in VVMD24 and VVIQ52) and 10 (in VMC8G9, UDV125 and VMC5G6.1). The expected heterozygosity (gene diversity) ranged from 0.63 at locus VVMD24 to 0.92 at locus VVMD28. The observed heterozygosity varied between 0.63 at locus VVMD24 to 1 at locus VVMD28 and was higher than the expected one at 4 out of 11 loci. Microsatellite analysis proved to be useful for the genetic characterization of Romanian grapevines, allowing precise identification and good discrimination of all tested cultivars.

Keywords: grapevine varieties, microsatellite, genetic characterization, SSR profile

Introduction

The grape is unique: not only is it a major global horticultural crop, but it also has ancient historical connections with the development of human culture. *Vitis vinifera* L. is the only specie from the *Vitaceae* family cultivated world-wide throughout the tropical and temperate regions and many important cultivars have been selected through the centuries. The inventory of grape cultivars described in the literature revealed the existence of more than 14000 putative cultivars. The dispersion of grape cultivars or populations of different origin by means of migration and trade led to a high number of synonymies, homonymies and ambiguities in cultivar identification which need to be solved. Studies of genetic diversity and genetic relatedness assisted by molecular markers can improve the use of the different genotypes in breeding programs and the design of new crosses. In addition, the DNA fingerprints generated can be very useful in certification programs to protect the new releases.

Microsatellites (or SSR – simple sequence repeats) consist of tandem repeated simple sequence motifs of 1 to 6 bp of DNA distributed throughout the genome of the Eukaryotes with a high variation in repeat number among individuals. The combination of several of their characteristics: abundance in the eukaryotic genomes, high level of polymorphism, Mendelian inheritance, co-dominance, locus-specificity, and PCR-based detection, have made them powerful genetic markers for humans, animals as well as for plants. Applications of microsatellite markers include individual or cultivar identification (HOKANSON & al. [1]), parentage testing (BOWLING & al. [2]), pedigree reconstruction (SEFC & al. [3]) and studies of population structure (MACHUGH & al. [4]; PETRI & al. [5]). These markers have recently proven to be useful in DNA fingerprinting and parentage analysis of grape cultivars

(SEFC & al. [6]). Indeed, their high rate of polymorphism provides unique genotypes for every distinct cultivar (THOMAS & al. [7]) and their codominant Mendelian inheritance allows the reconstruction of crosses (BOWERS AND MEREDITH [8]; SEFC & al. [9]; BOWERS & al. [10])

The intensive renewals of grapevine plantations, implementation of EU regulations and reshaping of national viticulture and wine industries that take place at present in Romania require application of efficient and reliable methods for the accurate cultivar identification. The aim of this study is to identify and discriminate eleven native Romanian grapevine varieties with the goal to obtain a genotype-specific profile by using microsatellites, the undisputed markers of choice for grape identification and parentage analysis.

Materials and Methods

Plant material (table 1) was obtained from two Grapevine Collections belonging to the Research and Development Station for Viticulture and Oenology Blaj and the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

Cuttings of 2-3 buds, each from individual vines, were collected during winter and kept in jars with water until budburst. DNA was extracted from young leaves collected from the shoot tips. They were frozen in liquid nitrogen and grounded to fine powder, used for DNA extraction following the DOYLE AND DOYLE [11] method modified by CIPRIANI AND MORGANTE [12].

Table 1. Grapevine cultivars investigated in this study

No	Cultivar	Utility	Berry colour
1	Timpuriu de Cluj	Table grape	White
2	Napoca	Table grape	Black
3	Transilvania	Table grape	Black
4	Cetățuia	Table grape	Black
5	Splendid	Table grape	Red-black
6	Blasius	Wine grape	White
7	Selena	Wine grape	Pink
8	Amurg	Wine grape	Black
9	Brumariu	Wine grape	White
10	Astra	Wine grape	White
11	Radames	Wine grape	Red-grey

DNA was quantified by visual comparison with lambda DNA molecular marker on ethidium bromide stained agarose gels and by fluorometry. DNA concentration varied between 63 and 185.5ng/µl. Extracted genomic DNA was amplified using eleven microsatellite markers previously developed for grapevine (table 2).

Polymerase chain reactions were carried out in a total volume of 25 µl containing 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of each primer, 1 unit of AmpliTaq Gold (PE Biosystems) and 50 ng of template DNA, using the following thermal profile: 95 °C for 2 min followed by 25 cycles at 94°C for 30s, annealing temperature for 45s (table 2) and 72°C for 1m, and a final extension step at 72°C for 7 min. PCR was repeated at least twice when bad or no amplification occurred at the first attempt.

We estimated several parameters for the evaluation of the microsatellite loci used. Allele frequencies, expected (He) and observed (Ho) heterozygosity, polymorphism information content (PIC) and mean number of alleles per locus were calculated by using the

Excel Microsatellite Toolkit (PARK [13]). The probability of null alleles (r) was estimated with the following formula (BROOKFIELD [14]): r= (He–Ho) / (1+He).

Table 2. Simple sequence repeats (SSR) markers assayed for the grapevine genotypes studied

SSR	Reference	Forward primer / Reverse primer	Annealing temperature
VVIP31	MERDINOGLU & al. [15]	TATCCAAGAGACAAATTCCCAC / TTCTCTTGTTTCCTGCAAATGG	59°C
VVIQ52	MERDINOGLU & al. [15]	TAAAAGGATGGTAGATGACAGA / ACAGGAAAGTGTTCAATGGTTA	58°C
VVIV37	MERDINOGLU & al. [15]	GGTAGACCTTGAAATGAAGTAA / ATGCTGAAGTCACGTAATAGAA	59°C
VVMD27	BOWERS & al. [10]	GTACCAGATCTGAATACATCCGTAAGT / ACGGGTATAGAGCAAACGGTGT	59°C
VVMD28	BOWERS & al. [10]	AACAATTCAATGAAAAGAGAGAGAGAGA / TCATCAATTTCGTATCTCTATTTGCTG	54°C
VVS2	THOMAS AND SCOTT [16]	CAG CCC GTA AAT GTA TCC ATC / AAATTCAAAATTCTAATTCAACTGG	59°C
VVMD24	BOWERS & al. [10]	GTGGATGATGGAGTAGTCACGC / GATTTTAGGTTCATGTTGGTGAAGG	54°C
UDV117	DI GASPERO & al. [17]	GGGTTTCCATCTCATTTTGTG / AAGTGTGGGCTTTGGAAATG	58°C
VMC8G9	DOLIGEZ & al. [18]	AACATTATCAACAACATGGTTTTA / ATATTCATCCTTCCCATCACTA	51°C
UDV125	DI GASPERO & al. [17]	GGCACTCCTAGATGATTTGTCC / TTCAGCTATGCACCGAGGTA	59°C
VMC5G6.1	RIAZ, S. & al. [19]	TTCTAAGACAGAATTGCTTGGC / TTATCTGTAGCTTTCACACCCC	51°C

Results and discusions

In our attempt to evaluate the degree of genetic polymorphism in some autochthonous Romanian grapevine varieties SSR markers were used already applied to characterise genetic resources, because they are robust markers that are readily shared among laboratories. Eleven highly polymorphic microsatellite markers were selected in order to genotype eleven grapevine cultivars, grown in Transilvania area.

All the primer pairs yielded clear and scorable amplified products and proved to be multi-allelic. The distribution of alleles for each locus allows to asses the identification ability of the markers. The most frequent alleles of the study were VVMD24 - 208 and VVIV37 - 164. Genetic parameters deduced from the results are shown in table 3. The total number of different alleles per locus ranged from 4 (in VVMD24 and VVIQ52) to 10 (in VMC8G9, UDV125 and VMC5G6.1) with a total of 87 alleles considering all loci and a mean value of 7.9 alleles per locus.

The microsatellite profile of the investigated cultivars showed a high level of genetic diversity among their flanking regions. Regarding the information content of the microsatellite markers, the most informative loci for the investigated set of cultivars were VMC8G9, UDV125 and VMC5G6.1 with 10 alleles. Less informative loci proved to be VVMD24 and VVIQ52 with 4 alleles. All cultivars were found to have a unique allelic profile as is presented in table 4. The number of alleles detected with Romanian grapevine genotypes

was very similar to those obtained with SSR microsatellites on European cultivars (SEFC & al. [20])

The expected heterozygosity (gene diversity) values were high for all DNA samples ranging from 0.63 at locus VVMD24 to 0.92 at locus VVMD28. The observed heterozygosity varied between 0.63 at locus VVMD24 to 1 at locus VVMD28 and was higher than the expected one at 4 out of 11 loci (Table 3). In contrast, it was lower than the expected values at loci VVS2, VVMD27, UDV117, VMC8G9 and UDV125. In consequence, the probability of occurrence of null alleles is positive at these loci.

Table 3. Genetic parameters obtained with 11 SSR markers for the 11 distinct cultivars.

Locus	Number of alleles	Expected heterozygosity	Observed heterozygosity	PIC	Probability of null alleles
VVIP31	9	0.90	0.90	0.84	0.00
VVS2	9	0.87	0.81	0.82	0.032
VVIV37	7	0.73	0.81	0.67	-0.046
VVIQ52	4	0.67	0.81	0.57	-0.083
VVMD24	4	0.63	0.63	0.54	0.00
VVMD27	7	0.83	0.72	0.76	0.060
VVMD28	9	0.92	1	0.87	-0.041
UDV117	8	0.80	0.72	0.74	0.044
VMC8G9	10	0.91	0.81	0.85	0.052
UDV125	10	0.85	0.72	0.79	0.070
VMC5G6.1	10	0.88	0.90	0.83	-0.010
Mean value	7.9	0.82	0.80		

Although these markers have already been evaluated in a set of cultivars, this is one of the first attempts to assess their information content in the Romanian grapevine population. Our results showed a large degree of genetic variability expressed as degree of heterozygosity. It was established that the high level of variability could be the result of natural cross-pollination and also the fact of humane selection for yield and quality. In the case of Romanian tested cultivars the mean expected heterozygosity was slightly higher (0.82) than the observed one (0.80) by a random union of gametes. Significant excess of the number of heterozygous individuals over Hardy-Weinberg expectations was observed at loci VVMD28, VVIV37 and VMC5G6.1.

The eleven microsatellite loci used amplified a high number of alleles and genotype. Hence, we may assume that the Romanian grapevine germplasm is characterized by a high genetic diversity at the DNA level. Other similar studies involving the analysis of microsatellites in grapevine have also detected loci with highly variable numbers of alleles (BOWERS & al. [21]; SEFC & al. [20]; ARADHYA & al. [22]; FATAHI & al. [23]).

Table 4. Genetic profiles of eleven Romanian grapevine cultivars analyzed at 11 microsatellite loci. Alleles size are given in base pairs.

Cultivar	VVIP31	VVS2	VVIV37	VVIQ52	VVMD24	VVMD27	VVMD28	UDV117	VMC8G9	UDV125	VMC5G6.1
Timpuriu de	181	156	151	81	206	180	220	150	175	136	150
Cluj	191	134	164	87	208	188	238	142		104	131
Napoca	201	142	162	81	202	202	218	141	169	104	134
		150	151		206	205	253	153		123	147
Transilvania	177	134	153	86	208	184	230	150	170	110	138
	191	136	164			193	247	167	173	116	146
Cetatuia	185	134	153	81	208	178	230	141	173	110	146
	187		164	87	212	184	261	150	170	152	138
Splendid	177	136	153	81	208	184	230	150	170	110	138
	191		164	87		193	247	167	173	116	146
Blasius	183	134	164	81	208	178	236	151	165	134	150
	197	146	172	87		180	246	167	186	140	155
Selena	195	146	164	81	208	184	246	150	176	134	155
	181	152		87	212	188	230		198		
Amurg	181	136	164	86	208	184	238	155	186	134	138
	191	140		79	212	188	246	142	208		141
Brumariu	187	134	155	87	206	180	253	150	176	110	131
	191	144	171	81	208		245	134	170	97	138
Astra	185	146	178	81	212	188	245	150	186	134	155
	197	144	164	87			261		202		138
Radames	181	152	164	81	208	188	236	155	175	130	163
	195	144	151	87	212		238		186	110	137

Conclusions

All data obtained in this study provide valuable genetic information, especially in the absence of comprehensive studies on the Romanian grape cultivars. SSR markers proved to be powerful tools and very efficient for cultivar identification and analysis of their genetic structure.

The eleven microsatellite markers chosen for this study proved to be informative and efficient for our study in Romanian grapevine population. All the cultivars were successfully distinguished with the selected SSR primers.

Genetic characterization of a larger number of autochthonous cultivars is the first step before to start further investigation to verify the homonymies and synonymies among Romanian cultivars and to reveal the genetic relationship between national and European grapevine germplasm.

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