Use of microsatellite markers to assess the identity and genetic diversity of *Vitis labrusca* and *Vitis rotundifolia* cultivars

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ABSTRACT. Ten grapevine cultivars were genotyped at eight microsatellite loci to characterize their identity and genetic diversity. Of these, nine cultivar profiles matched with those of databases and 'Magoon' matched with 'Regale' in the present study and 'Regale' in the University of California (Davis) database, implicating a likely error in planting. The number of alleles ranged from 5 (VVM5) to 9 (VVMD31), and the observed heterozygosity ranged from 37.14 (VVMD5) to 97.14% (VVMD27), with no significant differences in relation to the expected values for any of the loci, with the exception of VVMD5. The polymorphism information content values were observed to be above 0.25 in more than 85% of the loci analyzed, and VVMD31 was the most informative. The UPGMA analysis clustered the cultivars into two distinct groups. Within each group, the most divergent cultivars were 'Bountiful' (*V. rotundifolia*) and 'Goethe' (*V. labrusca*), also exhibiting the largest number of private alleles, 4 and 7, respectively. When comparing the two groups, the most divergent accessions were 'Bountiful' and 'Bordo', with the highest Nei distance. It was demonstrated that there is sufficient genetic variability in the cultivars used in this study to support breeding programs.

Keywords: grapevine, genetic variability, molecular marker.

Uso de marcadores microssatélites para acessar a identidade e diversidade genética de *Vitis labrusca* e *Vitis rotundifolia*

RESUMO. Dez cultivares de videira foram genotipadas com oito locos microssatélites a fim de confirmar a sua identidade e caracterizar sua diversidade genética. Destas, o perfil de nove cultivares foram idênticos aos da base de dados e o de 'Magoon' foi idêntico a 'Regale' deste estudo e de 'Regale' da base de dados da Universidade da Califórnia (Davis), indicando um provável erro de plantio. O número de alelos variou de 5 (VVM5) a 9 (VVMD31). A heterozigosidade observada variou de 37,14 (VVMD5) a 97,14% (VVMD27), sem diferenças significativas em relação aos valores esperados para todos os locos, exceto para VVMD5. Os valores de conteúdo de informação polimórfica (PIC) foram superiores a 0,25 em mais de 85% dos locos analisados, sendo que VVMD31 apresentou-se como o mais informativo. Por meio da análise de agrupamento UPGMA foi possível separar as cultivares em 2 grupos distintos. Dentro de cada grupo, as cultivares mais divergentes foram 'Bountiful' (*V. rotundifolia*) e 'Goethe' (*V. labrusca*), que também apresentaram o maior número de alelos privados, 4 e 7, respectivamente. Quando comparados os dois grupos, as cultivares mais divergentes foram 'Bountiful' e 'Bordo', com a maior distância de Nei. Foi evidenciado que há variabilidade genética suficiente nas cultivares utilizadas neste estudo para subsidiar programas de melhoramento genético.

Palavras-chave: videira, variabilidade genética, marcador molecular.

Introduction

Grapevine belongs to the *Vitaceae* family, comprising 19 genera. *Vitis* is the only genus that produces edible fruits and is divided into two subgenera or sections, namely *Euvitis* (bunch grapes) and *Muscadinia* (muscadine grapes). Muscadine grapes are genetically and morphologically distinct from the species within the subgenera *Euvitis*, and the most obvious genetic difference between these two taxa is the number of somatic chromosomes: *Muscadinia* species have 40, and *Euvitis* species have 38. *Muscadinia* species also differ from *Euvitis* species in their seeds, bark, tendrils and cluster morphology (OLIEN, 2001).

Euvitis species are divided into three geographical groups: American, Asian and European. *Vitis vinifera*, the main species currently cultivated worldwide, is the only member of the European group and has produced thousands of cultivars. The American group has approximately 30 species. Among these, *V. labrusca*, known for its 'foxy' fruit flavor, was widely used in the 18 and 19th centuries directly as rootstock for *V. vinifera* cultivars due to its phylloxera tolerance and in-crossings originating the so-called 'direct producer' vines. These hybrids were planted ungrafted and were bred to incorporate the resistance to the foliar diseases of the North American grapes with the fruit characteristics of the European grapevines (POMMER, 2003). *Muscadinia* is endemic to the southeastern United States of America, and, among the three species known, only *Vitis rotundifolia* Michx. is of commercial value, possessing a very strong resistance to grape pests and diseases, and is employed worldwide in breeding programs (OLIEN, 2001).

The knowledge of the genetic diversity of species has two advantages in a breeding program: the first concerns the genetic heterogeneity that limits the vulnerability of a species to pests and diseases; and the second is related to providing a large supply of allelic variation, which can be used for creating new combinations of favorable genes. The feasibility of using genetic diversity as a criterion for the selection of the parents for hybridization has been demonstrated in several species, including grapevine (OLIVEIRA et al., 2005).

DNA‑based molecular markers offer an advantage over morphological descriptors, as they are less prone to being affected by the environment or the developmental stage (CARIMI et al., 2010). Simple sequence repeat (SSR) markers have been increasingly used as molecular descriptors in grape. The usefulness of SSRs has been widely demonstrated in the identification and characterization of stock and rootstock varieties (DZHAMBAZOVA et al., 2007), evaluation of genetic variability (RIAZ et al., 2008; SCHUCK et al., 2009), pedigree studies (ORTIZ et al., 2004; VARGAS et al., 2009), and genetic mapping (RIAZ et al., 2004).

The development of a standard set of microsatellite markers for the identification of grape cultivars was proposed by the European Project GENRES 081 using six microsatellite loci (THIS et al., 2004). This set of markers, VVS2 (THOMAS; SCOTT, 1993), VVMD5, VVMD7 (BOWERS et al., 1996), VVMD27 (BOWERS et al., 1999), VrZAG62 and VrZAG79 (SEFC et al., 1999), was used by this project to evaluate 13 reference cultivars in Europe and are widely recommended by the International Organization of Vine and Wine for grapevine genotyping (OIV, 2007).

Considering that the knowledge about the genetic diversity of a specie movides subsidies to a breeding program, this study aimed to confirm the identity and estimate the genetic diversity among ten grapevine cultivars using eight microsatellite markers.

Material and methods

Plant materials

Vitis rotundifolia ('Bountiful', 'Carlos', 'Magnolia', 'Magoon' and 'Regale') and *Vitis labrusca* ('Bordo', 'Goethe', 'Isabel', 'Marta' and 'Niagara Rosada') cultivars (Table 1) from the grapevine germplasm collection of Estação Experimental do Canguiri, Universidade Federal do Paraná, Pinhais, Paraná State, Brazil, Estação Experimental de Videira, Videira, Santa Catarina State, Brazil, and Estação Experimental de Campos Novos, Campos Novos, Santa Catarina, State, Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri) were evaluated. These cultivars were selected due to their desirable agronomic characteristics and their potential for use as the parents in a breeding program.

Table 1. Description of the ten grapevine cultivars, type of flower, fruit color, species or parentage and geographical origin.

Cultivar	Flower type[1]	Fruit color[2]	Species or parentage	Geographical origin
			Vitis rotundifolia	
'Bountiful'	H	B	*Vitis rotundifolia*	USA
'Carlos'	H	Br	'Howard' x ('Topsail' x 'Tarheel')	USA
'Magnolia'	H	Br	('Thomas'x'Scuppernong') x ('Topsail' x 'Tarheel')	North Caroline
'Magoon'	H		'Thomas'x'Burgaw'	Mississippi
'Regale'	H	Bp	'Hunt' x 'Magnolia'	North Caroline
			Vitis labrusca	
'Bordo'	H	B	*V. labrusca* x *V. aestivalis* x *V. vinifera*	Ohio
'Goethe'	H	PR	'Muscat of Hamburg (Black Muscat) (*V. vinifera*) x 'Carter'	USA
'Isabel'	H	B	*V. labrusca* x *V. vinifera*	South Caroline
'Marta'	H	PR	*V. labrusca* x *V. vinifera*	USA
'Niagara Rosada'	H	PR	Somatic mutation of 'Niagara branca' ('Concord' and 'Cassady' *V. labrusca* x *V. vinifera*)	Jundiaí (SP)

[1]Hermaphrodite. [2]B=black; Br=bronze; Bp=black to dark purple; PR=pink-red.

SSR analysis

The DNA of the *Vitis labrusca* cultivars was analyzed in a previous study by Schuck et al. (2009).

The Genomic DNA of the *Vitis rotundifolia* cultivars was extracted using a modified CTAB (hexadecyltrimethylammonium bromide) procedure (RIAZ et al., 2004). In the final step, the DNA pellets were suspended in 100 μL 1X Tris‑EDTA buffer and stored at ‑20°C. The DNA quality was visualized on 1.2% agarose gels stained with ethidium bromide (10 mg mL^{-1}), and the samples were then diluted in ultrapure water to a final concentration of 10 ng μL^{-1}.

The eight microsatellite loci most frequently used by the international scientific community, VVS2 (THOMAS; SCOTT; 1993), VVMD27 (BOWERS et al., 1996), VVMD5, VVMD7, VVMD31, VVMD32 (BOWERS et al., 1999), VrZAG62 and VrZAG79 (SEFC et al., 1999), were used to genotype the samples.

The PCR reactions were performed in 10 μL reaction mixtures containing 5 pmol each primer, 2.5 mmol L^{-1} each dNTP, 1 μL 10X gold PCR buffer (Perkin Elmer Inc., Wellesley, MA, USA), 0.5 unit AmpliTaq Gold DNA polymerase (Perkin Elmer Inc., Wellesley, MA, USA), 2 mmol L^{-1} MgCl$_2$ and 10 ng genomic DNA. The temperature cycling for the PCR was performed using either a Peltier Thermal Cycler‑200 (MJ Research, Inc., Waltham, MA, USA) or a Bio‑Rad iCycler (Bio‑Rad Laboratories, Hercules, CA, USA). The following cycling program was used: denaturation of DNA and activation of Taq DNA polymerase at 95°C for 10 min.; 35 cycles of amplification consisting of 45 s at 94°C, 45 s at 56°C and 1 min. at 72°C; a final extension of 10 min. at 72°C; and cooling at 4°C. To separate the amplification products, the PCR reactions were mixed with denaturing dye (98% formamide, 10 mmol L^{-1} EDTA, 0.05% bromophenol blue and xylene cyanol) and heated at 94°C for 2 min. before loading onto 5% polyacrylamide sequencing gels. The gels were electrophoresed at a constant 70 W for 2–3 hours, depending on the allele sizes. The samples were visualized by silver staining using a commercial kit (Promega, Madison, WI, USA). All of the gels were visually examined and scored on a light box and were then digitally scanned to preserve the images.

To determine the size of the DNA fragments obtained by the amplification of the microsatellite products of the *V. rotundifolia* samples, aliquots of the *V. rotundifolia* DNA already diluted and available in the laboratory were loaded on the gels. These samples are part of the Grape Germplasm Collection of the University of California - Department of Viticulture and Enology, and the genetic profile in base pairs (bp) is in the reference database for the identification of grapevine that is maintained by the Foundation Plant Service, University of California, Davis, CA, USA. The genetic profile of *V. labrusca* was obtained in a previous study by Schuck et al. (2009) using the same microsatellite markers used in the present study (VVS2, VVMD5, VVMD7, VVMD27, VrZag62 and VrZag79).

Data analysis

Various genetic parameters for the ten cultivars over the eight SSR loci were calculated. The GDA v1.0 program package software (LEWIS; ZAYKIN, 2001) was used to calculate the allelic frequencies, number of alleles per locus (N), percentage of polymorphic loci (P), observed heterozygosity (H_o) and expected heterozygosity (H_e). The H_e values were estimated using the following formula (NEI, 1972):

$$H_e = 1 - \sum_{i=1}^{a} p_i^2 \ ,$$

where p_i is the frequency of the *i*th allele.

The GENES v2007.0.0 program package software (CRUZ, 2007) was used to determine the polymorphic information content (PIC). The PIC values were calculated according to Botstein et al. (1980), as follows:

$$PIC = 1 - \sum_{i=1}^{a} p_i^2 - \sum_{i,j=1 \, (i \neq j)}^{a} \sum p_i^2 p_j^2 \ ,$$

where p_i and p_j are the frequencies of the *i*th and *j*th allele. The probability of null alleles (*r*) was calculated for each microsatellite, as described by Brookfield (1996):

$$r = (H_e - H_o) / (1 + H_e) \ ,$$

where H_e is the expected heterozygosity and H_o is the observed heterozygosity.

The genetic distance between the cultivars were estimated as follows (NEI, 1972):

$$D = -\ln(I) \ ,$$

where ln is the natural logarithm and (*I*) is the measure of the genetic identity, as calculated using the following formula:

$$I = \frac{J_{PQ}}{\sqrt{J_P J_Q}},$$

where $J_P = \sum p_i^2$, $J_Q = \sum q_i^2$ and $J_{PQ} = \sum p_i q_i$, considering p_i and q_i as the frequencies of the i allele in the P and Q cultivars. Based on the matrix of Nei's (1972) genetic distance, a dendrogram was constructed using the Unweighted Pair Group Method Arithmetic Average (UPGMA). To verify the adjustment between the distance matrix and the dendrogram, a cophenetic correlation coefficient (r) was applied (SOKAL; ROHLF, 1962) using the GENES program package software (CRUZ, 2007).

Results and discussion

Among the five *V. rotundifolia* cultivars analyzed based on eight SSR loci, four different SSR profiles were detected (Table 2). 'Magoon' matched the same genetic profile of 'Regale' in the present study and the 'Regale' present in the Grape DNA Identification Reference Database, which is maintained by the Foundation Plant Service (University of California, Davis), indicating that the genotype was misnamed at the time of introduction and that the same genotype was planted under different names. The occurrence of misidentification is common, particularly for such old clonal species as *Vitis*, and it has been observed that 5 to 10% of the grape cultivars maintained in grape collections are incorrectly annotated (ANDRÉS et al., 2007). The identification of the *V. labrusca* cultivars was part of a previous study (SCHUCK et al., 2009), and the SSR profiles of 'Bordo', 'Goethe', 'Isabel', 'Niagara Rosada' and 'Marta' were the same as those available in the database for the same cultivars using the same SSR markers used in the present study. Therefore, these cultivars were correctly identified (Table 2).

Table 3 shows the alleles and their frequencies obtained for the different loci, with the allele sizes ranging from 118 bp (VVS2) to 301 bp (VVMD32). There were found important differences in the allelic composition, reflecting the existence of different sets of alleles for *V. rotundifolia* and *V. labrusca* and the existence of private alleles in each set. There were 23 alleles specific for the *V. rotundifolia* cultivars, 32 specific to *V. labrusca* and three alleles (246, 249 and 259 bp) common to the two groups. Private alleles were found in the *V. rotundifolia* cultivars Bountiful (4) and Carlos (3) and in the *V. labrusca* cultivars Niagara Rosada (2), Goethe (7) and Marta (1). The most frequent alleles were VVS2-147 and -149, VVMD5-230 and -234, VVMD7-235 and -243, VVMD31-201, VVMD32-249 and -301, and VrZAG62-199, showing frequencies greater than 20%. In contrast, 18 alleles were relatively infrequent (a frequency less than 10%). It is also worth noting that, of the alleles with a frequency higher than 20%, seven were unique to *V. rotundifolia*, further emphasizing the differences in the allelic composition of this species.

The alleles found in this study and in another study (RIAZ et al., 2008) indicate that the *V. rotundifolia* alleles are very different in size and frequency from those of *Euvitis* species. Riaz et al. (2008) reported the presence of specific alleles in a genetic diversity study of 57 accessions of *V. vinifera* and *V. rotundifolia* and a VR hybrid (*V. vinifera* x *V. rotundifolia*), including the loci VVMD7-245, VVMD27-197, -199 and -215, and VrZAG62-199 also used in our study. These authors also found that, of the total 184 alleles of 14 microsatellites loci used, 88 were specific to *V. rotundifolia*. In the present study, there was a relatively high number of alleles (39%) specific to the *V. rotundifolia* cultivars (Table 4); however, these alleles were common among the group of *V. rotundifolia* cultivars, suggesting that the genetic base of these cultivars is limited.

The main genetic parameters, such as the number of alleles per locus (N), percentage of polymorphic loci (P), observed (H_o) and expected (H_e) heterozygosity, estimated frequencies of null alleles (*r*) and polymorphic information content (PIC) for the eight SSR loci are shown in Table 4.

Table 2. Genetic profile of cultivars of *Vitis* spp. with the observed base par (bp).

Locos	*Vitis rotundifolia*					*Vitis labrusca*[1]				
	'Bontiful'	'Carlos'	'Magnolia'	'Magoon'	'Regale'	'Bordo'	'Goethe'	'Isabel'	'Marta'	'Niagara Rosada'
VVS2	153-153	147-149	147-147	147-147	147-147	118-130	120-130	118-146	120-146	118-128
VVMD5	230-246	230-246	230-230	226-230	226-230	234-234	230-236	236-236	234-234	234-234
VVMD7	235-235	243-245	243-245	243-245	243-243	235-239	235-247	235-249	235-249	235-241
VVMD27	199-199	197-215	185-215	185-215	185-197	179-181	181-183	175-179	179-181	175-181
VVMD31	246-250	168-170	170-210	170-210	170-210	201-213	203-209	201-213	201-203	201-201
VVMD32	249-301	249-301	249-301	249-301	249-301	248-248	246-252	248-272	248-248	248-272
VrZag62	215-223	199-215	199-215	199-199	199-199	200-202	190-204	200-202	200-202	200-202
VrZag79	255-255	257-287	259-287	255-259	255-259	247-247	239-247	237-247	247-264	237-259

[1]Cultivars analyzed by Schuck et al. (2009).

Table 3. Allele size (AS) and allele frequencies (AF) for 8 SSR markers evaluated among *V. rotundifolia* and *V. labrusca* cultivars.

VVS2		VVMD5		VVMD7		VVMD27		VVMD31		VVMD32		VrZag62		VrZag79	
AS	AF FA	AS TA	AF FA	AS TA	AF FA	AS TA	AF FA	AS TA	AF FA	AS TA	AF FA	AS TA	AF FA	AS TA	AF FA
118	0.15	226	0.10	*235*	0.35	*175*	0.10	168[2]	0.05	246	0.05	190[4]	0.05	*237*	0.10
120	0.10	230	0.35	*239*	0.05	*179*	0.15	170	0.20	248	0.20	199[1]	0.30	*239*[4]	0.05
128[3]	0.05	*234*	0.30	*241*[3]	0.05	*181*	0.20	*201*	0.25	249	0.25	*200*	0.20	*247*	0.15
130	0.10	*236*	0.05	243	0.30	*183*[4]	0.05	*203*	0.10	252[4]	0.05	*202*	0.20	255	0.20
146	0.10	_246_	0.10	245	0.10	185	0.15	209[4]	0.05	272	0.10	204[4]	0.05	257[2]	0.05
147	0.35			*247*[4]	0.05	197	0.15	210	0.15	301	0.25	215	0.15	_259_	0.20
149[2]	0.25			_249_	0.10	199	0.10	*213*	0.10			223[1]	0.05	*264*[5]	0.05
153[1]	0.10					215	0.10	246	0.05					287	0.10
								250[1]	0.05						

Alleles in italic are specific to *V. labrusca*, in bold are specific to *V. rotundifolia* and underlined are common between the two groups. Private alleles: 1-Bountiful, 2-Carlos, 3-Niagara Rosada, 4-Goethe, 5-Marta.

Table 4. Genetic parameters at the eight microsatellite loci, number of alleles per locus (N), percentage of polymorphic loci (P), observed (H_o) and expected (H_e) heterozygosity, estimated frequencies of null alleles (*r*) and polymorphic information content (PIC), calculated by grouping all of the cultivars into one group.

Loci	N	P (%) (%)	H_o (%)	H_e (%)	*r*	PIC
VVS2	8	100	0.8128	0.8286	0.0086	0.23
VVMD5	5	100	0.3714 [(1)]	0.7147 [(1)]	0.2002	0.19
VVMD7	7	100	0.8857	0.7901	-0.0529	0.26
VVMD27	8	100	0.9714 [(2)]	0.8356	-0.0739	0.34
VVMD31	9	100	0.8571	0.8559 [(2)]	-0.0006	0.34
VVMD32	6	100	0.7143	0.7557	0.0236	0.30
VrZag62	7	100	0.9143	0.7983	-0.0645	0.30
VrZag79	8	100	0.8286	0.8174	-0.0062	0.30
Total	58					
Mean	7.25	100	0.7944	0.7995		0.2825

[(1)]low value; [(2)]high value.

A total of 58 alleles were amplified, ranging from five (VVM5) to nine (VVMD31), with an average of 7.25 alleles per locus. These SSR loci have previously been used in the genetic characterization of other grape cultivars (LEÃO et al., 2009). Moreno-Sanz et al. (2008) analyzed the diversity among 46 accessions of *V. vinifera* and obtained a total of 37 alleles, with an average of 6.2 alleles per locus using six of the eight loci used in the present study. A larger number of alleles per SSR loci were observed in the *V. vinifera* cultivars (11) (MARTÍN et al., 2003), French-American hybrid cultivars (11) (POLLEFEYS; BOUSQUET, 2003) and table-grape cultivars (18) (IBÁÑEZ et al., 2009). In the case of the *V. vinifera* and table-grape cultivars, it is likely that the large number of cultivars analyzed (176 and 376, respectively) could account for the marginally higher number of alleles detected.

The expected heterozygosity (H_e) averaged 79.95%, ranging from 71.47% (VVMD5) to 85.59% (VVMD31). These values are higher than those reported by Salmaso et al. (2008) for 13 local northeastern Italian cultivars (20-79.6%) and Riaz et al. (2008) for *V. vinifera*, *V. rotundifolia* and *V. vinifera* x *V. rotundifolia* hybrids cultivars (5-85%) but similar to those found in the grapevine collections of Santa Catarina State, which are composed mainly of European, American and hybrid cultivars (SCHUCK et al., 2009).

The observed heterozygosity varied between 37.14% (VVMD5) to 97.14% (VVMD27), with a mean value of 79.44%, slightly higher than the corresponding expected heterozygosity for all of the loci, with the exception of the VVS2, VVMD5 and VVMD32 loci. The estimated frequency of null alleles (*r*) was negative for five out of eight loci and positive for each of those loci that showed $H_o < H_e$ and only high for VVMD5. For the VVMD5 locus, the H_e was high (71.47%), as with the other loci, but a very low H_o was observed (37.14%). For the other loci (VVS2 and VVMD32), the H_e value was slightly higher than the H_o value, and the frequency of null alleles was very low (Table 4). Nevertheless, the H_e and H_o values were high for these two loci. Such null alleles can arise when mutations prevent the primers from binding to the region. Cipriani et al. (2008) reported that a locus with a positive null allele value indicates an excess of homozygotes, but this does not necessarily imply that a null allele is present. Hence, the assumption of homozygosity rather than heterozygosity for a null allele could be considered adequate in the present study. Indeed, the highest number of homozygous cultivars in relation to the others was found for the VVMD5 locus (Table 2), a trend also observed by Martín et al. (2003). In the present study, the average value of observed heterozygosity (79.44%) was higher than that obtained for the *V. vinifera* (70.7%) (IBÁÑEZ et al., 2003) and *V. rotundifolia* (60%) (RIAZ et al., 2008) cultivars but is in the same range as that obtained when rootstock

cultivars are included in the analyses (ANDRÉS et al., 2007; THIS et al., 2004). Additionally, it was observed in the present work that the values of the expected and observed heterozygosity were greater than 70% for the majority of the loci (93.75%), indicating a high genetic variability of the markers analyzed. A high heterozygosity is commonly found in vegetatively propagated perennial species, such as grape (ARADHYA et al., 2003).

To characterize the usefulness of the eight SSR markers, the polymorphic information content (PIC) of each locus was assessed (Table 4). The PIC values were higher than 0.25 for more than 85% of the loci analyzed. The loci VVS2, VVMD27 and VrZAG79, which exhibited eight, seven and seven alleles, respectively, were also the most informative loci in other studies (MARTÍN et al., 2003). The number of alleles per locus, expected heterozygosity and polymorphic information content indicate VVMD31 as the locus with the highest information content (nine alleles, H_e = 85.59% and PIC = 0.34), whereas VVMD5 (PIC = 0,19) is the least informative. According to Botstein et al. (1980), markers with PIC values between 0.25 and 0.50 are considered moderately informative, and the markers chosen in this study are consistent with this criterion. The mean value of PIC (0,2825) and the number of alleles per locus (7,25) obtained in our study were lower than in previous studies: 0,782 and 11 (MARTÍN et al., 2003) and 0,777 and 7,8 (GONZÁLEZ-ANDRÉS et al., 2007), respectively. The authors of each study used six microsatellite loci, five (VVS2, VVMD5, VVMD7, VrZAG62 and VrZAG79) equal to those used in our study, to characterize the genetic diversity of 318 and 79 Spanish cultivars, respectively. The lowest number of alleles per locus and the PIC value for the loci used in this study can be attributed to the smaller number of cultivars analyzed (9).

A dendrogram depicting the genetic distance among the ten cultivars analyzed at eight loci was constructed using the UPGMA method (Figure 1). The cophenetic correlation coefficient (r) was 0.97, revealing a correlation between the dendrogram and the distance matrix. According to Sokal and Rohlf (1962), values of r ≥ 0.8 indicate a good fit between the original matrix and the cophenetic matrix values.

The dendrogram analysis identified two main groups: group I, containing the five *V. rotundifolia* cultivars ('Bountiful', 'Carlos', 'Magnolia', 'Regale' and 'Magoon'), and group II, with the five *V. labrusca* cultivars ('Bordo', 'Goethe', 'Isabel', 'Martha' and 'Niagara Rosada') (Figure 1). This result was expected because, in addition to the chromosomal differences, there are important distinguishing anatomical and morphological features between these species. For example, *Euvitis* species, such as *V. labrusca*, have a haploid number of 19 chromosomes, longitudinally shredding bark, lack lenticels on the stems, and possess a non-continuous pith that is segmented by diaphragms. In contrast, *V. rotundifolia* has 20 haploid chromosomes, adherent bark, prominent lenticels on the shoots and fruit, and a continuous pith lacking diaphragms (POMMER, 2003).

As expected, 'Regale' and 'Magoon' in the *V. rotundifolia* group presented a distance equal to zero (Figure 1). 'Magoon' showed the same genetic profile as the 'Regale' in this study and the 'Regale' present in the grape reference database (Table 2). The distance from 'Magnolia' to 'Regale' was 26%, and the distance from 'Carlos' to this group was 38.12% (Figure 1). 'Magnolia' shared 74% of its alleles with 'Regale', whereas 'Carlos' shared 67 and 62% of its alleles with 'Magnolia' and 'Regale', respectively (Table 2). These three cultivars have their geographic origin in North Carolina, and 'Magnolia' is also reported to be in the pedigree of 'Regale', showing the genetic similarity between these cultivars (RIAZ et al., 2008). 'Bountiful' was the most distant cultivar from all of the others in the same group, with a distance of 85.76% (Figure 1). Moreover, 'Bountiful' showed the largest number of private alleles within the *V. rotundifolia* group (Table 3).
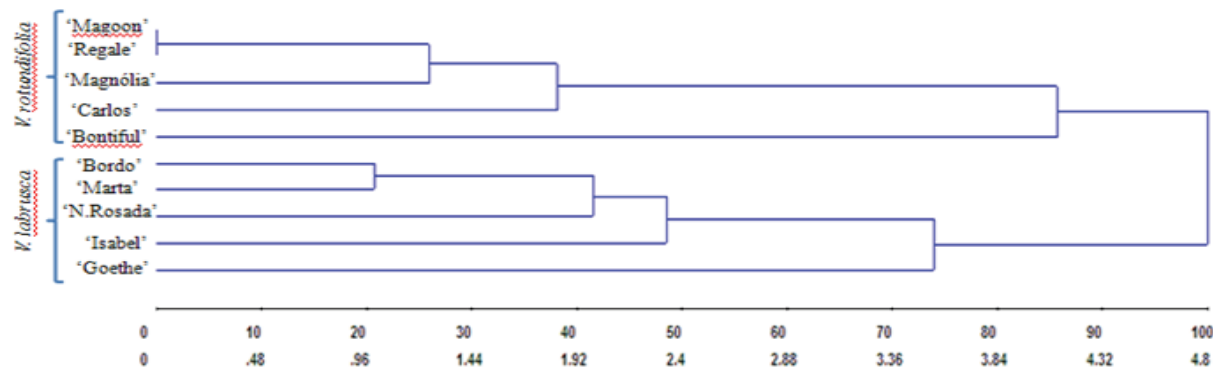
Figure 1. UPGMA dendrogram of ten grapevine cultivars using Nei's (1972) genetic distance matrix values.

In the *V. labrusca* group, 'Bordo' and 'Marta' were separated by a distance of 20.8% (Figure 1) and differed genetically only at four alleles (Table 2). 'Niagara Rosada' was separated from 'Bordo' and 'Marta' by a distance of 41.58%, and 'Isabel' was separated from this group by 48.51%. 'Goethe' was the cultivar most distant from all of the others of the same group, with a distance of 74.1% (Figure 1) and, within the *V. labrusca* group, was the most divergent cultivar and with the largest number of private alleles (Table 3).

The knowledge of the most divergent cultivars is essential to guide breeders in choosing the best combination for a cross. However, when selecting the parents, the agronomic characteristics of the genotypes and the purpose of the breeding program should be considered in addition to the genetic divergence. In a breeding program aimed at creating rootstocks, it is essential to choose cultivars with resistance and/or tolerance to biotic and abiotic stress. Regarding the cultivars characterized in this study, it is known that the *V. rotundifolia* cultivars are resistant to almost all of the pests and diseases of grapevine. It is worth highlighting the cultivars with resistance ('Bountiful' and 'Magnolia') and tolerance ('Regale') to the main soil pest of southern Brazil, ground pearl (BOTTON; COLLETA, 2010). The cultivars belonging to the *V. labrusca* group are well adapted to the vineyards of southern Brazil and display tolerance to *Fusarium oxysporum* f.sp. *herbemontis* (GARRIDO et al., 2004), making them an important genetic source for grapevine breeding programs.

Conclusion

SSR markers are efficient tools to confirm the identity and to assess the genetic diversity of *Vitis* cultivars.

The dendrogram clustered the ten grapevine cultivars in two groups, proving to be efficient in the separation of the cultivars based on their genealogy and botanical classification, showing relationships among cultivars of the same group.

The *V. rotundifolia* and *V. labrusca* groups have a high genetic diversity and can be used to support breeding programs.

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