Molecular characterization of grapevine from Santa Catarina, Brazil, using microsatellite markers

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Abstract – The objective of this work was to characterize the grape germplasm in Santa Catarina, Brazil, using microsatellite DNA markers (simple sequence repeats – SSR). The DNA samples were collected from leaves and shoots of accessions of public and private collections from the counties Urussanga, Nova Trento, Rodeio, São Joaquim, Campos Novos, Videira, and Água Doce. Ten SSR loci (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, VrZAG79, VVMD25, VVMD28, VVMD31, and VVMD32) were analysed by capillary electrophoresis. Molecular profiling was conducted for 190 grapevines (European, American, and hybrids), and 67 genotypes were obtained. The data were compared with each other and with those from the literature and from online databases, in order to identify varieties and discover cases of synonymy and homonymy. Forty molecular profiles corresponded to known varieties, while 27 genotypes were described for the first time. The existence of typical germplasm composed mainly of American and hybrid varieties is an important finding for local viticulture. Applications of the results rely on quality control and certification at the nursery level. Increasing precision in the characterization of grapevine genotypes may help breeding programs.

Index terms: *Vitis* spp., cultivar identification, genetic variability, germplasm collection, molecular markers.

Caracterização molecular de videiras de Santa Catarina por marcadores microssatélites

Resumo – O objetivo deste trabalho foi caracterizar a diversidade de videiras em Santa Catarina, por meio de marcadores moleculares microssatélites ("simple sequence repeats" – SSR). Amostras de DNA foram coletadas a partir de folhas e ramos de acessos de coleções de germoplasma públicas e privadas, nos municípios de Urussanga, Nova Trento, Rodeio, São Joaquim, Campos Novos, Videira e Água Doce. Dez loci SSR (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, VrZAG79, VVMD25, VVMD28, VVMD31 e VVMD32) foram analisados por eletroforese capilar. Foram produzidos perfis moleculares de 190 acessos de videira (europeus, americanos e híbridos), e 67 genótipos foram individualizados. Os dados foram comparados entre si e com aqueles disponíveis em literatura e em bancos de dados online, para a identificação de correspondências e casos de sinonímia e homonímia. Quarenta perfis moleculares corresponderam a variedades conhecidas, e 27 genótipos foram descritos pela primeira vez. A existência de um germoplasma típico, composto principalmente de variedades americanas e híbridas, é um fator importante para a viticultura local. A aplicação desses resultados poderá contribuir para o controle de qualidade e a certificação de mudas. Além disso, aumentar a precisão no que tange à caracterização genética da videira, auxiliará os programas de melhoramento genético.

Termos para indexação: *Vitis* spp., identificação de cultivar, variabilidade genética, coleção de germoplasma, marcadores moleculares.

Introduction

The Brazilian viticulture occupies 89.9 thousand hectares and yields about 1.3 million tons of grapes per year, concentrated in the south, southwest and northeast regions. The state of Rio Grande do Sul produces 90% of vines, juices and other grape products (IBGE, 2007).

The state of Santa Catarina produces the second largest crop, with approximately 50,000 tons of grapes per year. Wines are produced mostly from *Vitis labrusca*, an American species, or from hybrids adapted to the

environmental conditions of the state. Pinheiro Preto, Videira, Tangará, Iomerê, Caçador, Urussanga, Rodeio, and Nova Trento are the main grape-growing counties in Santa Catarina.

Since 1998, viticulture in Santa Catarina has undergone intense transformation, with the establishment of new vineyards of European grapes (*V. vinifera*). New viticultural areas have been established in the highland regions above 900 m, where climatic conditions determine specific features for grape maturation and wine specificity and quality. The most promising new areas are located in São Joaquim, Bom Retiro, Campos Novos, and Água Doce counties (Schuck et al., 2008).

Unlike the European market, where the "terroir" determines the quality of wines, the Brazilian market is based on the consumption of wines from a single variety. These are classified as regular wines, obtained from American and hybrid grapes, and fine table wines obtained from European white and black grape varieties, mainly Chardonnay, Cabernet Sauvignon, Cabernet Franc, Pinot Noir, and Merlot. The correct identification of grapevine germplasm is important since the expected quality of wines is related to characteristics of the cultivar.

In order to distinguish grape varieties, DNA-based markers offers an advantage over morphological descriptors, as they are less prone to being affected by the environment or the developmental stage (Sefc et al., 2001).

The available markers differ in the methodologies used to detect variability at the DNA level. Since 1990, simple sequence repeats (SSR) markers have been increasingly used as molecular descriptors in grape. Their usefulness has been widely demonstrated in the identification and characterization of stock and rootstock varieties, evaluation of genetic variability, pedigree studies, and genetic mapping (Bowers et al., 1999a; Pollefeys & Bousquet, 2003; Adam-Blondon et al., 2004; Riaz et al., 2004; Costantini et al., 2005). The analysis of grape plants with the aid of SSR markers allows identification profiles to be established and varietal reference data banks to be developed (Dettweiller et al., 1998; Lefort & Roubelakis-Angelakis, 2001; Grando et al., 2002). Access to data banks allows information to be shared and contributes to international cooperation to correctly identify grape germplasm.

The aim of this work was to characterize the diversity of the grape germplasm held in public and private collections in Santa Catarina, Southern Brazil, by means of SSR markers.

Materials and Methods

Leaves and shoots of 183 accessions were collected from 86 European, American and hybrid varieties, and another 7 accessions from unidentified sources (Table 1). The samples were taken from germplasm collections of different regions of Santa Catarina: families Quarezemin, Fellipi, Trevisol, Possamai, and Damiani (private collections) and Epagri (public collection) from Urussanga (28°31'4"S, 49°19'15"W, at altitude 49 m) and from Nova Trento (27°16'60"S, 48°55'0"W, at altitude 30 m); Vinicola San Michele from Rodeio (26°51'5"S, 49°19'60"W, at altitude 106 m); Terras Altas, Suzin, Villa Francioni, and Quinta da Neve (private collections) from São Joaquim (28°18'5"S, 49°55'60"W, at altitude 1,353 m); Epagri (public collection) from Campos Novos (27°23'60"S, 51°12'0"W, at altitude 947 m); Vinicola Pancieri (private collection), and Epagri (public collection) from Videira (27°0'5"S, 51°7'60"W, at altitude 750 m); and Fazenda Boa Esperança (private collection) from Água Doce (27°0'0"S, 51°32'60"W, at altitude 969 m).

DNA extraction was done according to Doyle & Doyle (1990), with the following modifications: freeze-dried material (instead of fresh material) was ground and RNase was added to isopropanol during the DNA precipitation phase. Quantification of the DNA extracts was performed in agarose gel (0.8%), and the samples were then diluted to a concentration of 20 ng μL^{-1}, at the Laboratório de Fisiologia do Desenvolvimento Genético Vegetal, of the Departamento de Fitotecnia, Universidade Federal de Santa Catarina.

In order to facilitate the comparison with data from the literature and international databases, the samples were analyzed at the ten microsatellite loci most frequently used by the international scientific community: VVS2 (Thomas & Scott, 1993), VVMD5, VVMD7 (Bowers et al., 1996), VVMD25, VVMD27, VVMD28, VVMD31, VVMD32 (Bowers et al., 1999b), VrZAG62 and VrZAG79 (Sefc et al., 1999).

Polymerase chain reaction (PCR) was carried out in a 12.5-µL total volume containing 25 mmol L^{-1} of each dNTP, 0.5 mmol L^{-1} of each primer, 0.5 U Taq DNA polymerase (GoldTaq – Applied Biosystem, Foster City, CA, USA or BioTaq – Bioline, London, UK), 1X buffer solution, 1.5 mmol L^{-1} $MgCl_2$, and 20 ng of genomic DNA for each sample. The cycling program used was: denaturation of DNA and activation of Taq DNA polymerase at 95°C for 7 min (GoldTaq) or

3 min (BioTaq); 35 cycles of amplification distributed in 45 s at 94°C, 45 s at 50°C (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, and VrZAG79) or 56°C (VVMD25, VVMD28, VVMD31, and VVMD32), 1 min and 30 s at 72°C; final extension of 7 min at 72°C; cooling at 4°C.

Capillary electrophoresis was used to determine the size of alleles. Separation of the microsatellite fragments was performed using the ABI 3100 sequencer and GeneScan 3.7 software (Applied Biosystems). The availability of primers labeled with different fluorescent dyes (Ned, Hex or Fam) enabled the product of different

Table 1. Grape accessions analyzed in this study.

Grape accessions	No. of samples	Cultivation region[(1)]	Grape accessions	No. of samples	Cultivation region[(1)]
Alicante Bouschet	1	V	Pinot Noir	3	AD, CN, SJ
Alphonse Lavallée	3	CN, V, SJ	Lorena	1	CN
Ancellota R2	1	AD	Isabel-SM	1	R
Barbera	1	SJ	Poloski	1	CN
Benitaka	2	CN, SJ	Luan Blanc	1	SJ
Bizaraqui – SM	1	R	Marta	1	NT, R, U
Bordô	6	NT, R, CN, U	Morena	1	V
Brasil	2	V	Primitivo	2	SJ
BRS Clara	1	CN	Red Globe	1	U
BRS Linda	1	CN	Red Meire	2	CN, V
BRS Morena	1	CN	Refosco	1	AD
Cabernet Franc	2	AD, SJ	Niágara Branca	2	CN, NT
Cabernet Sauvignon	20	CN, SJ	Niágara Rosada	1	CN
Carmenère	1	AD	Regente	1	U
Castelão	1	AD	Ora E	1	U
Catawba	2	U	Paulsen 1103	1	U
Centennial S	1	U	Renano	1	SJ
Chardonnay	4	AD, SJ	Pesc 2	1	U
Concord	2	CN	Rubi Itália	1	CN
Cristal	1	U	Sangiovese	5	AD, CN, R, SJ
Família Moscatel	1	U	Prima E	1	U
Fantasya	1	U	Rúbia	1	CN
Gamay	1	AD	São João	1	U
Gewürztraminer	1	AD	SO4	1	U
Goethe Clássica	9	U	Sauvignon Blanc	4	AD, CN, SJ
Goethe Primo	5	U	Vidal Blank	1	U
Gran D'oro	2	U, NT	Vilamar	1	U
Gravesac	1	U	VR 043-43	1	U
Gros Manseng	1	AD	101-14 Mgt	1	U
IAC 313	1	U	Tannat	1	AD
IAC 766	1	U	Tempranillo	3	AD, CN, SJ
Isabel	2	CN, NT	Teroldego	2	SJ, R
Isabel Precoce	1	CN	Tinta Roris	1	SJ
Itália	3	CN, V, SJ	Syrah	3	CN, SJ
Itália Export	1	CN	Tinturina	1	U
Itália Koga	1	CN	Touriga Francesa	1	AD
Lade	1	U	Touriga Nacional	1	AD
Magic Black	1	SJ	Traminer	1	SJ
Malbec	3	CN, V, SJ	Trincadeira	3	AD, SJ, R
Marcelan	3	V	Moscatel	1	R
Marzemino	1	AD	Viognier	1	V
Merlot	10	CN, SJ, R			
Montepulciano	3	AD, CN, R	NT4[(2)]	1	NT
Moscato EMPRAPA	1	CN	SJ12[(2)]	1	SJ
Moscato Giallo	1	CN	SJ13[(2)]	1	SJ
Muscat Alexandria	2	V	SJ14[(2)]	1	SJ
Mourvèdre	1	AD	SJ15[(2)]	1	SJ
Nebbiolo	2	AD, SJ	SJ17[(2)]	1	SJ
Petit Verdot	1	V	AD14[(2)]	1	AD

[(1)]AD, Água Doce; CN, Campos Novos; NT, Nova Trento; R, Rodeio; SJ, São Joaquim; U, Urussanga; V, Videira. [(2)]Accession number of unidentified plants.

PCR reactions to be loaded in multiplexes. Processing of the data generated by the sequencer was done with the Genotyper 3.7 program (Applied Biosystems). All tests were performed using DNA of the cultivar Chardonnay as a reference with respect to both the quality of amplification and the size of the amplified alleles, allowing the data to be compared with those available in the literature and in databases. This phase of the work was carried out at the Laboratory of Molecular Genetics of the Fondazione Edmund Mach – Istituto Agrario di San Michele all'Adige, in San Michele all'Adige, TN, Italy.

The molecular profiles obtained in this study were compared with online databases: Grape Microsatellite Collection (GMC) – http://www.ismaa.it/areabioav/gmc.html; Greek Vitis Database – (GVD) http://www.biology.uch.gr/gvd; Swiss Vitis Microsatellite Database (SVMD) – http:/www.unine.ch/nccr/svmd/; and GENRO – http://www.genres.de/eccdb/vitis/. Results were also compared to data from the literature not available online from the United States, Switzerland, Italy, Turkey, Armenia, and Georgia (Vouillamoz J., unpublished data). The latter databases currently contain details of microsatellites from more than 2,000 grapevine cultivars.

The Identity program (Wagner & Sefc, 1999) was used to calculate the number of alleles (n), the allele frequencies, expected (He) and observed (Ho) heterozygosity, the estimated frequency of null alleles (r), and the probability of identity (PI) at each locus. This software was also used to detect identical genotypes. Genetic similarity between the genotypes was calculated on the basis of the proportion of alleles in common (DPS), using the Microsat software (Minch et al., 1997). A neighbor-joining dendrogram was constructed from a matrix of genetic divergence (D) between the genotypes, calculated as D = 1-DPS, using Phylip (Felsenstein, 1989) and viewed with Tree View (Page, 1996).

Results and Discussion

The 190 grapevine accessions, belonging to 86 assumed varieties, generated 67 different profiles when analyzed at the 10 SSR loci. The reduction in the number of individual molecular profiles is due to synonymy, accessions identified with different names but having the same molecular profile. Seven synonymy groups were elucidated in this study: (1) Moscato Giallo, Moscato EMBRAPA, and Lorena; (2) Bordô, Grano d'Oro, and São João; (3) Goethe Primo, Goethe Clássica, and Moscatel; (4) Isabel, Isabel Precoce, and Bizaraqui-SM; (5) Itália, Itália Export, Itália Koga, Rubi Itália, Benitaka, Brasil, Redmeire, Lade, and Família Moscatel; (6) Niágara Branca, and Niágara Rosada; and (7) Ora E and Prima E.

Cases of synonymy could be clones of the same variety, which show phenotypic differences (Vignani et al., 1996; Walker et al., 2006). Despite these evident differences, they are not detectable by a low number of SSR markers, which are located in noncoding regions of the genome (Zulini et al., 2005). This was the case of 'Grano D'Oro', a mutation of the cultivar Bordô, which resulted in changes in vigor, productivity, and rusticity. This was also the case of 'Goethe Primo', a variant with white berries from 'Classic Goethe', which has pinkish berries.

Comparison of the data showed some cases of homonymy, that is, clusters of varieties having identical or similar names but different genotypes. This was the case of 'Isabel-SM' accession and the group of synonyms mentioned above, consisting of 'Isabel', 'Isabel Precoce', Catawba S, and Catawba T.

In addition, ten cases of misnaming were brought to light. An accession of 'Muscat of Alexandria' corresponded to the cultivar Sangiovese. 'Pinot Noir' collected in São Joaquim was actually 'Cabernet Sauvignon' and a 'Cabernet Sauvignon' accession of Campos Novos was identical to 'Carmènere'. The accession 'Luan Blanc' from São Joaquim and 'Tempranillo' from Água Doce were both 'Sauvignon Blanc'. Two accessions of 'Trincadeira' collected in São Joaquim and Rodeio corresponded to 'Pinot Noir'. The 'Tinta Roris' was identical to 'Tempranillo', and 'Trincadeira' from Água Doce was actually 'Viognier'. A 'Traminer' accession was wrongly named 'Gewürztraminer'.

Comparison between the molecular profiles from the ten SSR markers employed in the present work and those available in data banks allowed the recognition of 40 from the 67 distinct genotypes. They were mainly well-known international wine varieties. This comparison also allowed the identification of additional five cases of synonymy with known international varieties. Moreover, seven accessions without variety name in the collection were identified (Table 2).

The SSR profiles for rootstocks VR 043-43, 101-14 Mgt, Paulsen 1103, and SO4 did not correspond to the molecular characterization of the same rootstocks by Andrés et al. (2007). The possibility of errors in the

collecting procedures or in the introduction of these materials to the collections should be considered.

The remaining 27 molecular profiles did not correspond to those found in the literature, nor in the data banks (Table 3). The identification and description of unique molecular profiles is an important aspect of the regional viticulture. Nineteen genotypes were described for the first time in this study. Most of them are interspecific hybrids developed in Brazil. In other cases, are old varieties introduced during the first wave of Italian immigration to Southern Brazil at the end of the 19th century. Old varieties include the rootstock PESC 2 ("porta-enxerto Santa Catarina") from Rodeio, as well as the well-known 'Goethe' cultivar from Urussanga (Schuck et al., 2008). However, unique SSR profiles were also obtained from accessions thought to belong to international varieties as Catawba, Gros Manseng, and Paulsen 1103, which were determined to be not true-to-type.

Table 2. Comparison of the microsatellite profiles of the analyzed cultivars with those in the international literature and in online databases.

	Accessions	Correspondences
Synonymies	Niágara Branca/Niágara Rosada	Gros Framboise Blanc (Switzerland)
	Tinturina	Usellina (Switzerland)
	Castelão	Periquita (UC Davis)
	Magic Black	Exotic (Sanchez et al., 1999) and Cardinal (Switzerland)
	Renano	Riesling (UC Davis)
Identifications	AD14	Malbec
	SJ12	Pinot Noir
	SJ13	Cabernet Sauvignon
	SJ14	Cabernet Sauvignon
	SJ15	Syrah
	SJ17	Syrah
	NT4	Tinturina

Table 3. Smaller (SZ) and larger (LZ) allele sizes (bp) at ten microsatellite loci for the accessions analyzed in this study, without a genetic correspondence.

Accession	VVS2		VVMD5		VVMD7		VVMD27		VrZAG62		VrZAG79		VVMD25		VVMD28		VVMD31		VVMD32	
	SZ	LZ	SZ	LZ	SZ	LZ	SZ	LZ	SZ	LZ	SZ	LZ	SZ	LZ	SZ	LZ	SZ	LZ	SZ	LZ
Bordô	118	130	234	234	235	249	179	181	200	202	247	247	240	240	225	229	209	213	248	248
BRS Clara	128	130	224	236	239	253	175	191	186	186	247	255	240	240	217	225	209	213	252	272
BRS Linda	130	146	234	236	239	249	191	191	186	202	247	257	254	254	217	233	213	217	248	252
BRS Morena	130	130	234	236	239	249	175	175	186	194	255	257	240	256	217	233	209	213	264	272
Catawba S[1]	120	152	236	238	235	247	181	183	192	200	239	247	-	-	229	229	201	209	246	272
Catawba T[1]	118	130	238	238	235	247	165	181	192	200	239	247	242	256	229	229	201	209	246	272
Concord	120	128	234	234	235	241	181	181	200	204	247	259	240	240	229	243	185	199	250	272
Cristal	138	148	238	238	235	257	179	191	186	200	237	247	238	248	225	233	201	209	248	252
Fantasya	130	148	232	236	239	249	189	189	184	190	257	259	224	254	243	243	209	213	252	272
Goethe[1]	120	130	230	236	235	247	181	183	190	204	239	247	242	254	229	235	203	209	246	252
Gros Manseng[1]	134	134	232	238	237	239	185	185	192	192	251	251	240	250	227	233	213	213	240	240
Isabel – SM	120	136	234	234	235	241	179	181	200	200	247	259	242	242	225	229	201	201	252	252
Marcelan	134	140	224	230	243	247	175	191	190	202	255	257	240	242	233	243	203	207	240	240
Marta	120	146	234	234	235	249	179	181	200	202	247	264	250	250	225	229	201	213	248	248
Prima/Ora E	128	130	224	234	239	249	181	187	184	186	251	259	250	256	217	243	201	213	262	272
Poloski	138	138	230	234	237	243	177	177	178	186	255	255	240	250	233	243	207	211	256	256
Regente[1]	128	150	224	236	243	247	175	191	190	202	251	259	238	266	233	257	199	209	240	272
Rúbia	118	118	234	234	241	249	181	181	200	202	247	259	240	240	229	229	201	213	248	272
Vidal Blanc	128	146	226	230	237	247	177	185	184	186	251	259	238	238	233	243	201	211	256	272
Vilamar	128	140	236	242	239	249	175	191	186	194	259	261	242	256	229	243	201	201	252	272
IAC 313	132	138	238	238	245	251	183	207	188	190	257	257	240	240	219	253	203	209	240	240
IAC 766	128	132	230	236	235	235	195	203	198	198	243	255	240	240	213	251	197	197	238	238
Pesc 2	134	136	262	262	251	251	185	207	190	202	255	257	238	238	235	241	197	203	238	246
SO4[1]	140	144	234	234	239	253	185	185	186	192	245	257	238	246	251	251	-	-	260	260
VR 043-43	138	142	226	248	235	249	181	191	200	202	247	264	248	266	243	243	203	209	244	272
101-14 Mgt[1]	132	140	250	264	239	251	181	185	186	192	259	261	238	252	213	243	197	209	236	238
Paulsen 1103[1]	132	144	234	264	257	265	181	183	190	204	239	247	236	246	-	-	201	209	260	260
Chardonnay[2]	132	138	232	236	239	243	177	185	186	194	243	245	238	254	216	227	210	214	239	271

[1]Not true-to-type. [2]Reference variety.

The number of alleles, the expected and observed heterozygosity, the estimated frequency of null alleles, and the probability of identity with the 67 genotypes are listed in Table 4. The germplasm analyzed in this study showed wide variability in the size of SSR alleles and encompassed most (79.9% mean) of the known allelic variants (This et al., 2004). Some alleles commonly present in European varieties were observed, while others had the characteristics of American or hybrid varieties (Figure 1).

The expected heterozygosity, in the 10 evaluated loci, ranged from 0.798 to 0.889, these values being higher than those observed by Sefc et al. (2000) in European varieties (0.677-0.819), but similar to those found in Spanish varieties and Franco-American hybrids (Martín et al., 2003), and in American hybrids cultivated in the USA (Pollefeys & Bousquet, 2003). These results were expected since the grape germplasm from Santa Catarina encompasses European and American varieties, as well as different hybrids.

The observed heterozygosity ranged from 0.687 to 0.849, with values lower than those expected from randomized union of gametes (H_e) in all loci, except for VVMD7. For these loci, the probability of null alleles was positive, suggesting that most of the apparent homozygotes could be heterozygotes, one allele being visible and the other not. These types of null alleles can occur, when mutations do not allow the linking of primers to the target region (Pollefeys & Bousquet, 2003).

In eight out of ten analyzed loci, the probability of identity was higher than 0.05, and their use in combination resulted in an accumulated probability (the probability of obtaining by chance two identical profiles, when belonging to distinct varieties) of $2.13x10^{-12}$, thus confirming the high degree of polymorphism in the chosen SSR markers.

The different molecular profiles were also evaluated to visualize similar groups to check whether such groups coincide with the known history of the varieties. The dendrogram (Figure 2) shows three main differentiated groups, which suggests three different origins for the grape germplasm in Santa Catarina. Group 1 includes European varieties of French origin, such as Cabernet Franc, Cabernet Sauvignon, Carmènere, Merlot, Sauvignon Blanc, Pinot Noir, and Chardonnay. Group 2 is composed by a small number of accessions, mainly Italian varieties, such as Sangiovese, Ancellota, Syrah, Marzemino, Refosco, Nebbiolo, and Teroldego. Finally, the third and largest group comprises almost exclusively American and hybrid varieties, such as Prima/Ora, BRS Clara, BRS Morena, Centennial, BRS Linda, Fantasya, Isabel, Isabel SM, Bordô, Marta SM, Concord Precoce, Niagara, Rubia, Cristal, VR 043-43, Paulsen 1103, Goethe, Catawba S, Catawba T, IAC 766, IAC 313, Gravesac, PESC 2, RR 101-14 Mgt, and SO4, most of them developed in Brazil.

The results of the present work corroborate the empirical and historical origin of grape germplasm in Santa Catarina. The recent expansion and renovation of viticulture in this state was based on the introduction of European germplasm, as shown by data from groups 1 and 2. Group 3, however, is predominantly composed of well-established and recently introduced American and hybrid grape varieties, which are still a valuable source of income for small farmers in Santa Catarina.

Table 4. Genetic parameters of the ten microsatellite loci analyzed with 67 grapevine varieties[(1)].

Locus	n	H_e	H_o	r	PI
VVS2	17	0.889	0.821	+0.036	0.040
VVMD5	12	0.850	0.758	+0.050	0.075
VVMD7	14	0.848	0.849	-0.001	0.069
VVMD27	14	0.849	0.687	+0.088	0.072
VrZAG62	11	0.846	0.803	+0.023	0.076
VrZAG79	12	0.875	0.846	+0.015	0.053
VVMD25	13	0.798	0.631	+0.093	0.107
VVMD28	19	0.886	0.754	+0.070	0.044
VVMD31	10	0.813	0.727	+0.047	0.105
VVMD32	15	0.845	0.731	+0.062	0.073
Total	137	-	-	-	$2.13x10^{-12}$
Mean	13.7	0.850	0.761	-	0.071

[(1)]n, number of alleles; H_e, expected heterozygosity; H_o, observed heterozygosity; r, expected frequency of null alleles; PI, probability of identity.

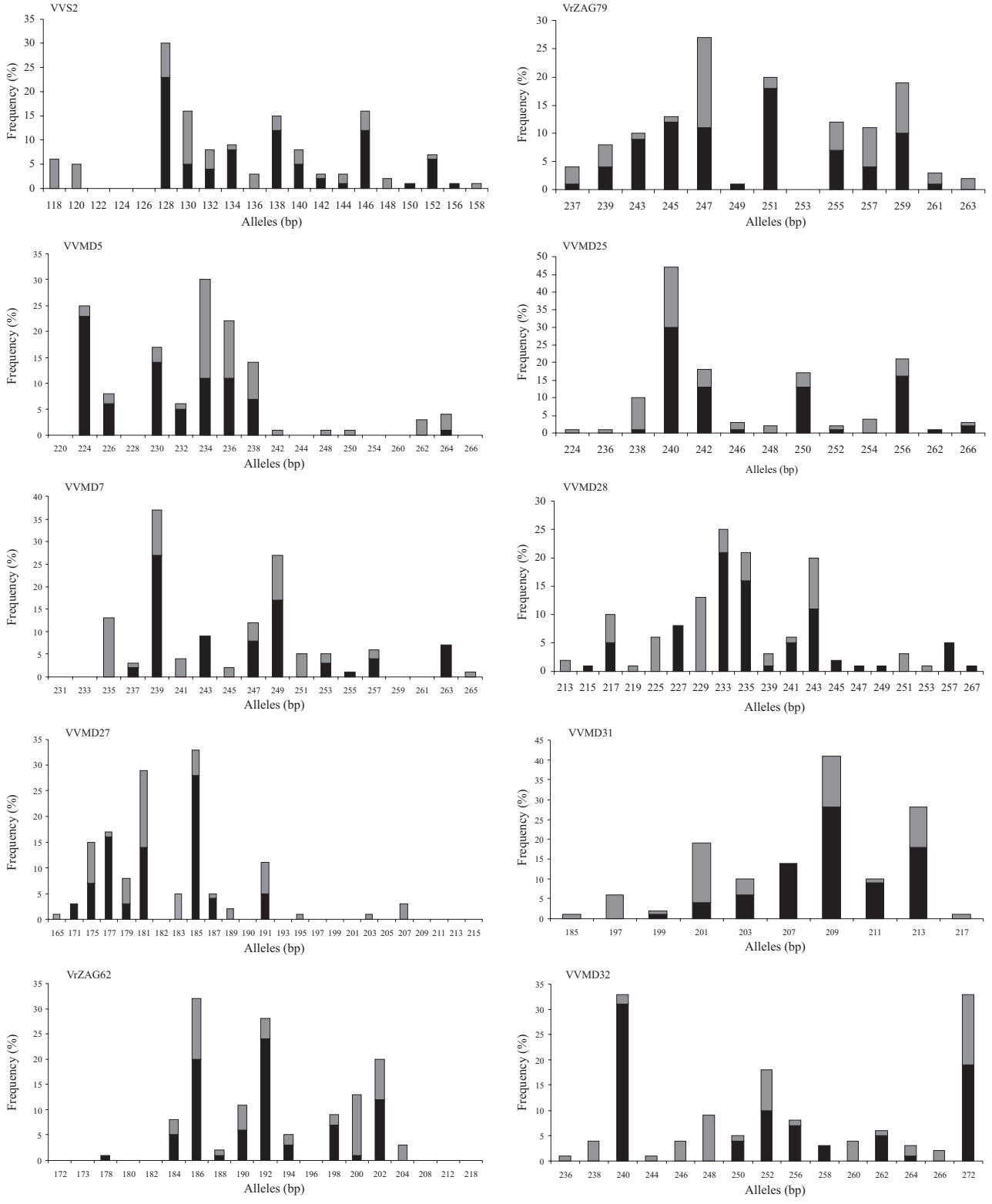

Figure 1. Distribution and relative frequency of microsatellite alleles in 67 grape cultivars from Santa Catarina, Brazil, compared with known variability in the genus *Vitis* at six microsatellite loci (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, and VrZAG79), and at four microsatellite loci (VVMD25, VVMD28, VVMD31, and VVMD32) (■ European cultivar, ■ non-European cultivar).

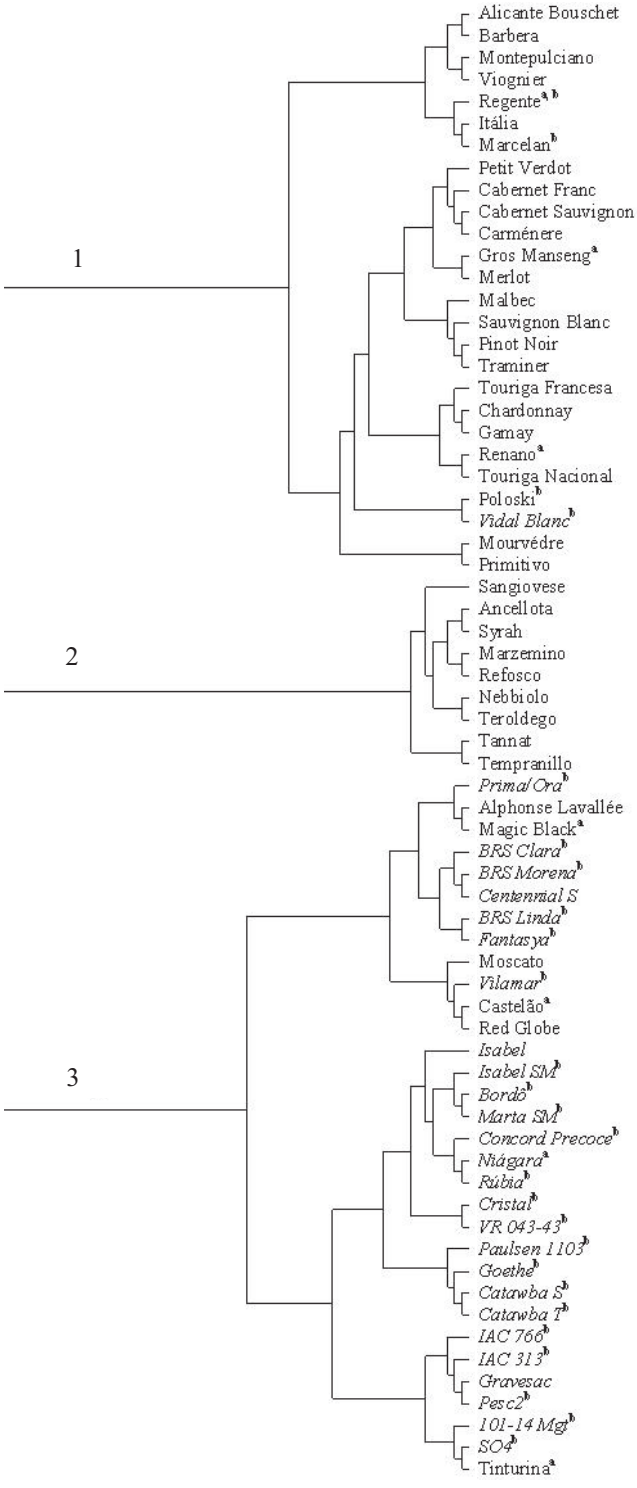

Figure 2. Dendrogram of the genetic relationships among the 67 grapevine varieties investigated in this study, calculated with proportional shared alleles (DPS) (Bowcock et al., 1994) genetic distance. Non-European varieties are indicated in italics. [(a)]Not true-to-type. [(b)]Without genetic correspondence.

Conclusions

1. Genotype analyses using ten simple sequence repeat (SSR) loci was adequate to identify the grape germplasm in use in Santa Catarina.

2. The genotypes in these collections were mostly European varieties, but some American and hybrid varieties were also present.

3. The existence of unique regional germplasm was supported by the finding that several SSR genotypes did not match any molecular profile in the literature and databases.

4. Taken together the results can be used to better rationalize and manage grape collections, as well as for providing reference DNA profiles for the nursery industry and for breeding.

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