Characterization of a Brazilian Grape Germplasm Collection Using Microsatellite Markers

Patricia C.S. Leão,^{1*} Summaira Riaz,² Rachel Graziani,² Gerald S. Dangl,³ Sergio Y. Motoike,⁴ and M. Andrew Walker²

Abstract: Two hundred and twenty-one grapevine accessions from the Embrapa Semi-Árido, Juazeiro, Bahia, collection in Brazil were fingerprinted at seven SSR loci: VVS2, VVMD5, VVMD7, VVMD27, VVMD31, VrZAG62, and VrZAG79. Of these, 187 accessions had reliable allelic profiles allowing them to be divided into three groups. Group 1 consisted of 86 accessions that were correctly identified; group 2 consisted of 30 accessions that were incorrectly named, but matched the reference profile of a different cultivar; and group 3 consisted of 71 accessions with SSR profiles that did not match any available reference profile. Group 3 contained 11 accessions that did not match their internationally validated reference and 60 accessions for which international reference profiles did not exist. The profiles of group 3 may then serve as references for those accessions. The SSR allelic profiles from the reported parents of 19 of the group 3 accessions were used to determine whether the accessions were correctly named and six were confirmed. These profiles can now serve as references for this group of important Brazilian cultivars.

Key words: Vitis, SSR markers, parentage, genetic diversity

Brazil is the largest country in South America and has a wide range of climatic conditions, resulting in a wide diversity of plants adapted to the tropical rainforests in the Amazon, Atlantic forests along the coast, savannahs in the central-west region, and semiarid areas in the northeast. Grapevines are cultivated in Brazil from the extreme south (33°S) to the northeast (7°S), with modified horticultural techniques adapted to each region (Pommer et al. 2000). There are ~76,987 ha of grape in Brazil (Mello 2007), with production centered in the states of Rio Grande do Sul and São Paulo. Breeders of new table grapes and winegrapes are selecting varieties tolerant of extreme climatic conditions and adapted to the required horticultural practices, which include the ability to produce two crops per year with high-quality fruit. Most grapes grown in Brazil are European varieties (Vitis vinifera L.) or American hybrids. Several hybrids from breeding programs in Brazil have also been successful.

¹Embrapa Semi-Árido, BR 428 km 152, Zona Rural, Caixa Postal 23, Petrolina, CEP. 56.302-970 Brasil; ²Department of Viticulture and Enology, ³Foundation Plant Services, University of California, Davis, CA 95616; ⁴Departamento de Fitotecnia, Universidade Federal de Viçosa, Av. Peter Henry Rolfs, s/n, Viçosa, CEP. 36.570-000, Brasil.

Acknowledgments: The authors gratefully acknowledge research support from Embrapa Semi-Árido, Universidade Federal de Viçosa (UFV), and the Fundacao Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior (CAPES) program. Additional support from the UC Davis Louis P. Martini Endowed Chair in Viticulture funds.

Manuscript submitted Nov 2008, revised May 2009, accepted Jun 2009. Publication costs of this article defrayed in part by page fees.

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There are three major grapevine germplasm repositories in Brazil: National Grape Germplasm Repository at Embrapa Uva e Vinho, Jales, São Paulo; Instituto Agronômico de Campinas, São Paulo; and Embrapa Semi-Árido, Juazeiro, Bahia. These collections preserve diverse grape germplasm, provide support to grape-breeding programs, facilitate the exchange of germplasm between diverse institutes within Brazil, and provide support to growers. The Embrapa Semi-Arido grapevine germplasm collection, consisting of 230 accessions, was established in 1963 and provides support specifically to the grape industry in the São Francisco Valley. Many of these accessions are suitable for cultivation in the tropical semiarid conditions of northeastern Brazil. Although some of these studied accessions were from Embrapa Uva e Vinho and Instituto Agronômico de Campinas, the origin of many accessions is unknown.

Accurate identification of accessions is a basic requirement for coherent management of germplasm repositories and the use of the germplasm in ongoing breeding programs. It is essential to identify and correct mistakes resulting from the initial misnaming of accessions at the time of introduction and to identify the existence of multiple synonyms for one cultivar and errors in subsequent propagation. DNA-based microsatellite markers have been used in conjunction with traditional ampelography to resolve such issues at many national grape germplasm collections: Spain (Sanchez-Escribano et al. 1999, Ibáñez et al. 2003, Núñez et al. 2004, Martín et al. 2006, Yuste et al. 2006, Fernández-González et al. 2007), Portugal (Lopes et al. 1999, 2006, Almadanim et al. 2007), Italy (Grando and Frisinghelli 1998, Constantini et al. 2005), Austria (Sefc et al. 1998), Iran (Fatahi et al. 2003), Croatia (Maletic et al. 1999), Greece (Lefort and Roubelakis-Angelakis 2001), United States (Lamboy and Alpha 1998, Dangl et

^{*}Corresponding author (email: patricia@cpatsa.embrapa.br)

al. 2001), Peru and Argentina (Martínez et al. 2006), and Chile (Narváez et al. 2001). In Brazil, however, there is no information available regarding the use of reliable DNA-based marker technology to manage grape germplasm collections.

In the present study a standard set of microsatellite DNA markers (This et al. 2004) was used to generate profiles (DNA fingerprints) for 221 grapevine accessions maintained at Embrapa Semi-Árido, including table grape and winegrape scions, rootstocks, Brazilian breeding selections, and *Vitis* species. The resulting profiles were compared to one another and to existing databases of grape DNA profiles. This is the first use of microsatellite DNA markers to characterize germplasm from a Brazilian grapevine collection.

Materials and Methods

Plant material. Two hundred twenty-one accessions from the grapevine germplasm collection at Embrapa Semi-Árido, Juazeiro, Bahia, were analyzed. Young leaves from each accession were collected and dried between sheets of absorbent blotting paper with silica gel packet in sealed envelopes. Eight to 10 envelopes were packed into one resealable plastic bag with 50–60 g Drierite crystals. Bags were kept sealed and stored in a cool, dark place.

DNA extraction. Dried leaf tissue was homogenized with DNA extraction buffer in plastic bags using a Homes 6 mechanical homogenizer (Bioreba, Longmont, CO). Genomic DNA was extracted using a modified CTAB (hexadecyltrimethyl-ammonium bromide) protocol (Lodhi et al. 1994). In the final step, DNA pellets were suspended in 100 μL 1x Tris-EDTA buffer and stored at -20°C.

Microsatellite analysis. Seven well-characterized, highly polymorphic grapevine SSR markers were used: VVS2 (Thomas and Scott 1993), VVMD5, VVMD7, VVMD27, VVMD31 (Bowers et al. 1996, 1999), and VrZAG62 and VrZAG79 (Sefc et al. 1999). One primer in each primer pair was 5'-labeled with one of the following fluorescent dyes: 6-FAM, HEX, and NED. PCR amplification were performed separately for each locus in a 10 µL final volume containing 2.5 ng/µL template DNA, 10 pmole each primer, 2.5 mM each dNTP (Applied Biosystems, Foster City, CA), 1 µL 10x Gold PCR buffer (Applied Biosystems), 2 mM MgCl₂ (Applied Biosystems), and 0.5 units Ampli-Taq Gold DNA polymerase (Applied Biosystems). PCR reactions were carried out using a PTC-100 thermalcycler (MJ Research, Waltham, MA). The cycling program for all markers consisted of the following steps: 5 min at 95°C, followed by 35 cycles of 30 sec at 95°C, 45 sec at 60°C, 1 min at 72°C, and a final extension step of 7 min at 72°C. Amplifications were checked by running an aliquot of 4 µL of the PCR reaction product on ethidium bromide stained 2% agarose gels. Aliquots (1.7 μL) of PCR products were mixed with 0.7 µL formamide, 0.4 µL dye, and 0.2 µL DNA size standard (GeneScan 500 ROX, Applied Biosystems). Samples were denatured at 94°C for 2 min, and loaded onto an ABI 377 DNA sequencer (Applied Biosystems).

To obtain consistent and reliable results, each sample was amplified and analyzed twice at each marker. Four grape cultivars with well-established SSR profiles (Chardonnay, Carignane, Riesling, and Thompson Seedless) were loaded on each gel for consistent scoring from one gel to another. The PCR fragments were detected with GeneScan analysis software version 3.1 and the alleles were scored using the Genotyper DNA fragment analysis software version 2.5.2. (Applied Biosystems).

Data analysis. The SSR fingerprints were compared to previously published profiles (Sánchez-Escribano et al. 1999, Sefc et al. 1999) and three reference databases: the Grape DNA Identification Reference Database (Foundation Plant Services, University of California, Davis); the Greek Vitis databases (http://gvd.biology.uoc.gr/gvd/contents/index.htm); and the Swiss Vitis Microsatellite database (http://wwwl.unine.ch/svmd/). The allele sizes were first standardized to be consistent with various references (This et al. 2004).

Results and Discussion

Two hundred twenty-one grapevine accessions in the grape germplasm collection of Embrapa Semi-Árido, Juazeiro, were genotyped at seven SSR loci. Consistent and reliable profiles were obtained for 187 accessions at all SSR markers. Thirty-four samples were excluded due to collection errors, poor DNA quality, or inconsistent profiles between two independent runs. The 187 reliable allelic profiles were compared to available reference databases. The results allowed the collection to be divided into three distinct groups: group 1, accessions with profiles that match references of the same name; group 2, accessions with profiles that match references of a different name; and group 3, accessions with profiles that did not match any available reference profile.

There were 86 accessions in group 1 with SSR profiles that are identical at all seven loci to a validated reference profile of the same or essentially the same name of an accepted synonym (Table 1). The results also confirmed matches to reference profiles of clones based on somatic mutations. Differences in SSR profiles among clones of a cultivar are rare, although differences in SSR profiles have been detected (Riaz et al. 2002, Hocquigny et al. 2006, Moncada et al. 2006). In this study, the five clones of Italia have the same SSR profile, although they have significant viticultural differences. Itália clone 1, known as Italia melhorada, in the São Francisco Valley has larger berries and clusters, leading to higher yields and more pronounced muscat flavor compared to other clones of Italia. The clusters of Itália clone 2 are more conical with larger shoulders, and the clone Italia Muscat has a more intense muscat flavor. The clones Benitaka and Brasil are berry color mutants with red and black berries, respectively, and are grown as distinct cultivars in Brazil (Leão et al. 2001). There were also cases of clear synonyms. The SSR profile data confirmed Tinta Roriz as a synonym for Tempranillo and Sultanina Branca as a synonym for Thompson

	Table 1 Eighty-six access	ions from the Embrapa S	emi-Árido collection	that are accurately identifie	ed (group 1).
n	Matches ^a	Comments	Accession	Matches ^a	Comments

Accession	Matchesa	Comments	Accession	Matchesa	Comments
101-14	VR	Millardet de Grasset 101-14	Madeleine Royal	VR	
1613C	VR	Couderc 1613	Malvasia Branca	VR	
420A	VR	Millardet de Grasset 420A	Moscato Canelli	VR	Muscat blanc, Muscat a Petits
Alfrocheiro	VR			• • • •	Grains
Altesse	VR		Moscato de Alexandria	VR	Muscat of Alexandria
Ancellota	VR		Moscato de Hamburgo	VR	Muscat Hamburg
Barbera	VR		Mouverdre	VR	Mataro, Mourvedre, Esparte
Baresana	VR	White Tokay	Neo Muscat	VR	
Beauty Seedless	VR		Niagara Rosada	Clone	Niagara
Benitaka	Clone	Italia	Olivet noir	VR	Cornichon
Brasil	Clone	Italia	Palomino	VR	Listan
Bronx Seedless	VR		Panse Precoce	VR	
Burger	VR	Monbadon	Paulsen 1103	VR	
Cabernet Sauvignon	VR		Periquita	VR	Castelao
Campanário	VR		Perla de Gralia	VR	Perle de Csaba, Carrière
Cardinal	VR		Peverella	VR	Verdicchio
Carignane	VR		Queen	VR	
Catalunha	VR	Thompson Seedless	Red Vletliner	VR	Veltliner rouge
Centennial Seedless	VR		Redglobe	VR	· ·
Chenin blanc	VR		Regina de Vignetti	VR	Scolokertek kiralynoje, Queen of the Vineyard
Crimson Seedless	VR		Riesling Itálico	VR	, , , , ,
Delight	VR		Royalty	VR	
Dogridge	VR		Ruby Cabernet	VR	
Emerald -	VR		Ruby Seedless	VR	
Emperor	VR		Saint Jeannet	VR	Gros Vert, Rosaki, Verdal
Fantasy seedless	VR		Sangeovese	VR	Sangiovese
Fiesta	VR		Seara Nova	VR	Canglevede
Flame Seedless	VR		Semillon	VR	
Flora	VR		Seyve Villard 20365	VR	Dattier di Saint Vallier
Gamay	VR	Gamay noir	Siegerrebe	VR	Dattier di Saint Vallier
Gewürztraminer	Clone	Traminer	Souzao	VR	
Grand noir	VR		Suffolk Red Seedless	VR VR	Suffolk Red
Grenache	VR			VR VR	
Gros Golman	VR		Sultanina Branca		Thompson Seedless
Himrod Seedless	VR		Sultanina Moscato	VR	2
Imperio	VR	Imperator	Superior Seedless	VR	Sugraone
Isabel	VR		Sylvaner	VR	Sylvaner blanc
Itália	VR		Tannat	VR	
Itália clone 1	Clone	Italia	Tempranillo	VR	Tinta Roriz, Valdepenas
Itália clone 2	Clone	Italia	Thompson Seedless	VR	
Italia Muscat	Clone	Italia	Tibouren	VR	Tibouren noir
Kober 5BB	VR		Tinta Roriz	VR	Tempranillo
Lakemont Seedless	VR		Tocai Fruilano	VR	Tocai Friulano, Sauvignonasse
Loose Perlette	Clone	Perlette	Ugni blanc	VR	Trebbiano Toscano, St. Emilion

^aVR: accession that matches a validated reference SSR profile of the same cultivar or an accepted synonym. Clone: accession with an SSR profile that indicates it is a clonal form of a given cultivar.

Seedless. Catalunha was also confirmed as a synonym for Thompson Seedless.

Group 2 consists of 30 accessions with SSR profiles that match the validated reference profile of a different prime name (Table 2). Based on a match at all seven loci, the collection names of these 30 accessions can be corrected. The two accessions of Petit Syrah are included in this group. In California, Petite Sirah is an important winegrape capable of producing very dark, full-bodied wines. It is now known to be the French cultivar

Durif (Peloursin x Syrah) and was occasionally confused with Peloursin in older California vineyards (Meredith et al. 1999). In this study, the profiles of the two accessions of Petite Syrah match the profile of Syrah, clearly indicating a naming error in the collection. There were other cases where the names of collection accessions could be corrected: Kagina is Cardinal; Sauvignon is Cabernet Sauvignon; Branca Salitre is Gros Vert; Ceilad and Portuguesa Blanes are Criolla Mediana; Piratininga is Queen; and Trebbiano Toscano is Sauvignonasse (Table 2).

Group 3 consists of 71 accessions with profiles that did not match any available reference profile and includes cultivars from grape-breeding programs in Brazil (Instituto Agronômico de Campinas and Embrapa), Argentina (Gargiulo-Instituto Nacional de Vitivinicultura), United States (Arkansas, Florida, and New York), and France (Seyve Villard series). The largest subset of breeding program accessions are from Brazilian programs at the Instituto

Agronômico de Campinas (IAC) (A Dona, Aurora or IAC 77526, Isaura, Juliana, Patricia, Paulistinha, and the rootstocks IAC 313, IAC 572, and IAC 766) and the Embrapa Uva e Vinho (BRS Clara, BRS Linda, BRS Lorena, BRS Morena, BRS Rubea, and Moscato Embrapa). Eleven accessions in group 3 did not match the internationally validated reference profiles for the same name and are therefore incorrectly identified; however, the correct name can only be determined for several of these accessions (Table 3). Although Orange Muscat does not match its international reference profile, it does match the profile of both IAC 77526 and Aurora. IAC 77526 is the breeder's selection code for Aurora; thus these are two separate samples of the same cultivar. The matching profiles strongly suggest Aurora and IAC 77526 are both correctly identified and that these SSR profiles can be used as a reference. There are several other internal matches between accessions (Table 3). The correct names for Mgt 41B, Isabel Precoce, and Mission from the collection may be IAC 313, BRS Lorena,

Table 2 Thirty accessions from the Embrapa Semi-Árido collection with names that were corrected by comparing SSR profiles with validated references (group 2). In some cases synonyms for the reference name are noted under comments.

Accession	Matches correct name	Comments
99 Richer	Richter 110	
Baco blanc	Semillon	
Blush Seedless	Black Monukka	
Branca Salitre	Gros Vert	Rosaki, Saint Jeannet, Verdal
Canner	Thompson Seedless	
Ceilad	Criolla Mediana	Black Prince
Christmas Rose	Emperor	
Cinsaut	Syrah	
Dattier de Beiroth	Darkaïa	
Dawn Seedless	Centennial Seedless	
Deckrot	Pinot noir	
Estevão Marinho	Black Morocco	
Frankenthal	Cornichon violet	Alulu, Rosa Minna
Gamay Beaujolais	Valdiguié	
Kagina	Cardinal	
Malvasia Chartrense	Valenci blanco	Teneron, Beba, Malaga
Mont Serrat	Exotic	
Moscato Grega	Chasselas blanc	There are several berry color clones with the same profile
Moscato Rosada	Malaga Rosada	Moscatel Rosada De Blas
Muscat caillaba	Muscat Hamburg	Muscat caillaba is synonym for Muscat blanc
Piratininga	Queen	
Petit Syrah FR	Syrah	
Petit Syrah RS	Syrah	
Portuguese Blanes	Criolla Mediana	Black Prince
Regina Roma	Dattier de Beyrouth	Karabournov, Waltham Cross
Rodi	Perlette	
Rosaky Rosada	Cornichon violet	Alulu, Rosa Minna
Sauvignon	Cabernet Sauvignon	
Tardia de Caxias	Riesling	
Trebbiano Toscano	Sauvignonasse	Tocai Friulano

and IAC 766, respectively. However, more morphological and source information is needed to validate their identification and their use as reference cultivars.

There are 60 accessions for which international reference profiles do not exist (Table 4). There are several internal matches confirming that CG 28467 and Emperatriz are the same, in addition to Aurora and IAC 77526 mentioned above. However, the profiles of Jupiter and CG 26858 match as do A1105 and A1581, thus one or both are incorrectly identified in the collection. The SSR profile of the cultivars Dona Maria, Marroo Seedless, and Moscatuel (cultivar name of CG 102295) are the same as those observed elsewhere (Sánchez-Escribano et al. 1999) for the same cultivars using SSR markers VVS2, VVMD5, and VVMD7, indicating that these cultivars are correctly identified.

Few of the breeding program accessions have international reference profiles, but they do have published pedigrees that allow comparison of the allelic profile of the accessions to the profile of the known parents. The selections from the University of Arkansas (A1105, A1581, A1118, Reliance, Saturn, and Venus) had high degree of genetic similarity. A1105 and A1581 have the same allelic profile across the seven loci; both are seedless grapes but have white and dark purple colored fruit, respectively. These cultivars could be siblings or perhaps share a clonal relationship; more marker data and pedigree information is needed to clarify the relationship.

The origin and pedigree of the accession Roni Redi is unknown, but it shares one allele at six loci with Beni Fugi, which is a tetraploid and a cross of Golden Muscat x Kuroshio. SSR profile data was only available for Golden Muscat, and although SSR data were consistent at six loci, given the tetraploid nature of Beni Fugi more SSR data is needed to validate its parentage.

The SSR profiles for Baviera, Dacari, Damarim, Emperatriz, and Moscatuel confirm that they are synonyms

Table 3 Eleven accessions from the Embrapa Semi-Árido collection with SSR profiles that do not match validated reference profiles and are incorrectly identified (from group 3). Internal match refers to multiple accessions of the same genotype in the collection.

3 71 -	
Accession	Internal match
Mgt 41B	IAC 313
Colombard	
Dattier Saint Vallier	
Early Muscat	
Ferral	
Isabel Precoce	BRS Lorena
Mission	IAC 766
Muller Thurgau	
Orange Muscat	Aurora, IAC 77526 ^a
Petit Verdot	
SO4	

^aIAC 77526 is selection number of Aurora.

for the numbered selections CG 26916, CG 102024, CG 40016, CG 28467, and CG 102295, respectively. These cultivars were developed by Gargiulo at INTA-Argentina. The pedigrees of CG 26916, CG 28467, and CG 87908 were not available; however, they shared one allele across six loci with Thompson Seedless, suggesting that this latter cultivar is in their pedigree.

The German cultivar Regner shared one allele at all seven loci with Seyve Villard 12375, strongly suggesting a parental/offspring relationship. Thus we can conclude this accession is misnamed in the collection, because Regner is a pure *V. vinifera* cultivar (Luglienca Bianca x Early Gamay; Vitis International Variety Catalog).

The allelic profiles of 19 accessions from group 3 were compared to the reference database profiles of one or both of the reported parents. The indirect analysis results based on pedigree are shown (Table 4). The accessions Angelo Pirovano, BRS Rubea, CG 33716, Feal, and Ferlongo did not share profile for one or both parents, suggesting they are incorrectly identified in the collection. Morphological traits in the field confirmed the molecular profile results of two accessions. BRS Rubea has red berries, but the plants in the collection have white berries (Camargo and Dias 1999), confirming the SSR profile mismatch. Ferlongo is a Portuguese grape with black berries (Vitis International Variety Catalog), although the sampled plant had white berries. In addition, its profile matched that of Moscatel Nazareno, thus the collection's Ferlongo is actually Moscatel Nazareno. It was not possible to compare the profiles of seven accessions (Juliana, Lake Emerald, Mars, Paulistinha, Sovrano Pirovano, Tampa, and Venus) with the profiles of both reported parents (Table 4).

Parentage analysis did confirm the identity of six cultivars (BRS Morena, CG 26858, CG 38049, Marroo Seedless, Moscatel Nazareno, and Reliance), allowing the SSR profiles presented here to be used a valid reference (Table 4). The profiles of CG 26858 and Jupiter matched, thus this latter cultivar from the University of Arkansas must be misidentified in the collection.

Germplasm management requires attention at many stages, including the integrity of origin (from other collections or from nondocumented locations), correct identification and passport data, propagation of plant material, and field planting records. Mistakes happen at each stage of the introduction and maintenance of new accessions. In addition to naming errors, the existence of synonyms and homonyms is a major challenge to the management of germplasm collections. Eleven SSR markers were used to profile a grape germplasm collection in Portugal and several cases of synonyms and homonyms were identified (Lopes et al. 1999, 2006). In Spain, synonyms and homonyms were detected when accessions from different collections and regions were examined (Ibáñez et al. 2003, Yuste et al. 2006, Fernández-González et al. 2007). Nine cases of synonyms and seven cases of homonyms were identified when 114 accessions were analyzed from the Campania region of Italy with eight SSR markers (Costantini et al. 2005).

 Table 4
 Sixty accessions from the EMBRAPA Semi-Árido, Juazeiro, BA collection with SSR profiles that do not match a validated reference profile.

 Allele sizes (bp) for seven SSR markers are listed. Internal Match refers to multiple accessions of the same genotype in the collection.

 Cases where SSR profiles could be used to confirm parentages and therefore verify accession identity are noted.

Accession Us	Use	Parentage	Internal match/ parentage confirmed	VVMD5	VVMD7	VVMD27	VVMD31	VVS2	VRIP62	VRIP79
A1105	 -	Hybrid	A1581	236:238	239:247	179:185	212:216	125:151	197:205	251:265
A1118	—	Hybrid		226:238	239:251	179:185	216:216	133:151	195:197	251:255
A1581	⊢	Hybrid	A1105	236:238	239:247	179:185	212:216	125:151	197:205	251:265
A Dona	—	Soraya × IAC 544-14 ^b	Himoront	232:238	241:253	179:194	212:212	133:151	189:203	255:255
Ângelo Pirovano	—	Chasselas Rose x Muscat Hamburg	Unconfirmed	234:236	239:247	185:185	210:210	135:143	189:193	239:245
	≯	(IAC 394-16 × Maria) × Moscatel branco ^b	IAC77526	234:248	237:249	181:181	204:204	149:149	183:187	249:255
Baviera	—			234:234	239:243	181:194	212:214	143:151	189:189	243:259
Beni Fugi	-	Golden Muscat × Kuroshio°		238:238	239:249	185:185	204:212-	133:135- 149	187:189	ND: ND
Blue Lake	-	V. smalliana O.P. × Caco⁰		226:232	235:237	189:189	206:214	133:133	181:207	247:261
Bordo	≥	Hartford × O.P.°		236:236	235:249	185:185	204:216	123:135	203:203	247:247
BRS Clara	-	CNPUV 154-147 × Centennial Seedless ^d		226:238	239:253	179:194	212:216	133:133	189:189	247:255
BRS Linda	—	CNPUV 154-90 × Saturnd		236:238	239:249	194:194	216:220	135:151	189:205	247:257
	≥	Malvasia Bianca × Seyval⁴	Isabel Precoce	232:236	235:247	187:187	204:212	125:133	195:203	237:247
BRS Morena	-	Marroo Seedless × Centennial Seedless⁴	Confirmed	236:238	ND: ND	177:192	212:216	135:135	189:197	255:257
BRS Rubea	_	Niagara Rosada × Bordo⁴	Unconfirmed	226:252	239:243	179:189	212:212	133:133	189:189	255:261
California		V. vinifera		228:232	239:247- 249	183:185	212:212	133:135- 143	189:193- 195	237:243
CG 26858 T	Д,	Alphonsee Lavallee × Sultanina°	Confirmed	234:238	253:255	185:194	210:210	135:145	189:205	239:259
CG 28467	⊢	Emperor × Sultanina ^c	Emperatriz	234:236	243:253	194:194	210:212	135:145	189:189	257:259
CG 33716	—	Dattier de Beiroth × Thompson Seedless°	Unconfirmed	236:236	239:243	189:194	210:210	135:145	189:189	243:259
CG 351	—	Gibi × Sultanina°		234:234	237:245	181:181	210:212	133:143	189:189	ND:ND
CG 38049	≥	Riesling × (Gibi × Sultanina)⁵	Confirmed	234:234	239:249	189:194	212:214	151:151	189:205	245:257
CG 39915	—	Saint Jeannet × Sultaninac		234:246	239:247	185:194	212:212	143:143	187:189	251:259
CG 87908	—	V. vinifera		228:236	239:247	181:181	212:216	145:151	189:205	241:247
Dacari				226:228	249:253	181:194	212:216	149:149	187:189	247:247
Damarim				228:234	239:253	181:181	212:212	145:145	189:189	247:255
Dominga	۳,۲	V. vinifera		234:238	249:251	183:194	210:220	133:135	189:205	247:257
	L, W	Moscatel de Setubal × Rosaki⁰		226:228	239:251	185:194	212:224	135:149	189:205	247:251
Emperatriz	—	Emperor × Sultanina ^c	CG 28467	234:236	243:253	194:194	210:212	135:145	189:189	257:259
Feal	⊢	Italia × Fernão Pires∘	Unconfirmed	226:228	239:239	192:192	212:212	145:149	189:189	245:245
	—	Ferral × Alphonse Lavallee°	Mos. Nazareno	236:238	243:249	179:179	204:216	145:149	187:189	251:255
Himoront	⊢	Soraya × IAC 544-14♭	A Dona	232:238	241:253	179:194	212:212	133:151	189:203	255:255
	RS	Golia × <i>V. cinerea</i> ⁵	Mgt 41B	240:268	235:257	ND:ND	200:218	143:143	197:205	243:255
	RS	Mg 106-8 × <i>V. tiliifolia</i> ⁰	Mission	240:240	235:265	199:207	200:200	133:137	201:205	243:255
IAC 77526	≥	(IAC 394-16 × Maria) × Mos. branco ^b	Aurora	234:248	237:249	181:181	204:204	149:149	183:187	249:255
Isaura	—	Hybrid		232:238	243:249	179:185	210:212	143:149	187:189	255:259
Juliana	-	Italia × Madalena (IAC 21-14)⁵	Unconfirmed	228:238	239:247	179:179	204:212	137:149	189:193	243:255
Jupiter	—	A 1258 × A 1672°	CG 26858	234:238	253:255	185:194	210:210	135:145	189:205	239:259
Lake Emerald M	Τ,ν	Pixiola x Golden Muscat ^c	Unconfirmed	238:238	237:249	179:185	216:216	141:149	181:187	239:249
0	—	Pirovano 50 × Delizia de Vaprioº		226:232	239:249	183:194	210:222	133:133	189:205	247:257
Marroo Seedless	-	Carolina Blackrose x Ruby Seedless°	Confirmed	236:236	239:249	194:194	212:220	135:151	197:205	257:257

Mars	-	Island belle × A1339°	Unconfirmed	236:244	235:249	183:185	204:216	125:151	187:203	247:247
Moscatel Nazareno	≥	Muscat Hamburg $ imes$ Joao Santarem $^\circ$	Confirmed	236:238	243:249	179:181	204:216	145:149	187:189	251:255
Moscato Embrapa	≯	Hybrid		238:240	235:251	185:185	196:210	141:151	195:203	239:251
Moscato noir	T,W	V. vinifera		228:236	ND: ND	185:185	218:222	135:149	187:193	251:251
Moscatuel	⊢	Moscatel Rosada n°2 × (Cardinal × Sultanina) $^\circ$		226:228	239:249	181:181	210:210	135:149	187:189	247:255
Muscat Saint Vallier	-	Seyve Villard 12-129 × Panse		232:238	239:249	179:189	212:212	135:149	187:189	255:259
Neptune	⊢	Hybrid		238:238	233:237	185:194	198:212	143:149	193:201	251:265
Patricia	-	Soraya × IAC 544-14⁵		232:236	235:247	179:199	204:214	133:151	193:205	243:247
Paulistinha	-	Niagara Branca × Sultanina⁵	Unconfirmed	234:234	235:239	179:194	ND:ND	123:151	189:205	237:259
Perlona	⊢	Bicane × Muscat Madresfield ^c		238:238	243:247	179:194	202:210	133:135	189:193	255:255
Regner	≯	Luglienca Bianca × Gamay Precoce°		226:232	247:251	181:189	214:214	143:151	195:195	245:255
Reliance	Ľ,W	Ontario × Suffolk Red ^c	Confirmed	236:238	235:247	185:185	204:216	125:155	203:205	247:259
Roni Redi				232:238	235:249	185:185	204:204	125:135	187:203	239:243
Saturn	⊢	Dunstan 210 × New York 45791 ^c		238:238	239:247	179:185	212:216	133:151	197:205	251:265
Seyve Villard 12327	>	Seibel 6468 × Seibel 6905°		226:232	237:239	181:181	198:214	135:145	181:195	243:255
Seyve Villard 12375	≥	Seibel 6468 × Seibel 6905°		232:236	237:251	181:189	214:214	133:143	181:195	255:261
Sovrano Pirovano	-	Frankenthal × Delizia de Vaprio°	Unconfirmed	232:236	247:249	181:194	212:224	133:135	193:205	257:259
Stover	W,T	Mantey × Roucaneuf⁵		232:236- 283	235 : 243- 249	179:189	204:216	133:145- 149	187:207	251:255
Tampa	RS	V. smalliana O.P. × Niagara∘	Unconfirmed	248:248	235:241	185:194	204:204	133:137	173:203	247:259
Venus	⊢	Alden × New York 46000°	Unconfirmed	238:238	235:235	185:185	196:216	125:135	203:207	265:265
aW. winegrape T. tabl	ים מראה פי	aW. winegrane T. table grane B. raisin BS. rootstock . It grane in ice								

⊌W: winegrape, T: table grape, R: raisin, RS: rootstock, J: grape juice. ⁵Instituto Agronômico de Campinas (www.iac.sp.gov.br/centros/fruticultura/melhoramento%20videira.htm).

Embrapa Uva e Vinho (http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Uva/UvaAmericanaHibridaClimaTemperado/cultivar.htm#cultivares) Vitis International Variety Catalogue (http://www.vivc.bafz.de/index.php) Database (www.genres.de/eccdb/vitis/) European Vitis

Conclusion

This study generated a SSR profile database for the accessions from the grape germplasm collection of Embrapa Semi-Árido. The results permitted the integration of fingerprint profile data with morphological characteristics to establish the accurate identification of cultivars, identify naming errors, and identify a set of unique accessions that do not match cultivars in international reference databases. This last set of accessions is very important and will greatly influence current and future breeding programs in Brazil. The results from this study are also important to nurseries and the grape industry and for the legal protection of the cultivars that were developed from Brazilian breeding programs.

Literature Cited

- Almadanim, M.C., M.M. Baleiras-Couto, H.S. Pereira, L.C. Carneiro, P. Fevereiro, J.E. Eiras-Dias, L. Morais-Cecilio, W. Viegas, and M.M. Veloso. 2007. Genetic diversity of the grapevine (*Vitis vinifera* L.) cultivars most utilized for wine production in Portugal. Vitis 46:116-119.
- Bowers, J.E., G.S. Dangl, and C.P. Meredith. 1999. Development and characterization of additional microsatellite DNA markers for grape. Am. J. Enol. Vitic. 50:243-246.
- Bowers, J., G.S. Dangl, R. Vignani, and C.P. Meredith. 1996. Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome 39:628-633.
- Camargo, U.A., and M.F. Dias. 1999. BRS Rubea. Comunicado Técnico Nº. 33, pp. 1-4. Embrapa Uva e Vinho, Bento Gonçalves, Brazil.
- Costantini, L., A. Monaco, J.F. Vouillamoz, M. Forlani, and M.S. Grando. 2005. Genetic relationships among local *Vitis vinifera* cultivars from Campania (Italy). Vitis 44:25-34.
- Dangl, G.S., M.L. Mendum, B.P. Bernard, M.A. Walker, C.P. Meredith, and C.J. Simon. 2001. Simple sequence repeat analysis of a clonally propagated species: A tool for managing a grape germplasm collection. Genome 44:432-438.
- Fatahi, R., A. Ebadi, N. Bassil, S.A. Mehlenbacher, and Z. Zamani. 2003. Characterization of Iranian grapevine cultivars using microsatellite markers. Vitis 42:185-192.
- Fernández-González, M., A. Mena, P. Izquierdo, and J. Martínez. 2007. Genetic characterization of grapevine (*Vitis vinifera* L.) cultivars from Castilla la Mancha (Spain) using microsatellite markers. Vitis 46:126-130.
- Grando, M.S., and C. Frisinghelli. 1998. Grape microsatellite markers: Sizing of DNA alleles and genotype analysis of some grapevine cultivars. Vitis 37:79-82.
- Hocquigny, S., F. Pelsy, V. Dumas, S. Kindt, M.C. Heloir, and D. Merdinoglu. 2006. Diversification within grape-vine cultivars goes through chimeric states. Genome 47:579-589.
- Ibáñez, J., M.T. de Andrés, A. Molino, and J. Borrego. 2003. Genetic study of key Spanish grapevine varieties using microsatellite analysis. Am. J. Enol. Vitic. 54:22-30.
- Lamboy, W.F., and C.G. Alpha. 1998. Using simple sequence repeats (SSRs) for DNA fingerprinting germplasm

- accessions of grape (Vitis L.) species. J. Amer. Soc. Hort. Sci. 123:182-188.
- Leão, P.C.S. 2001. Principais cultivares de uvas finas de mesa. *In* Uva de mesa produção: Aspectos técnicos. P.C.S. Leão (ed.), pp. 26-33. Embrapa Informação tecnológica. Embrapa Semi-Árido, Brasília.
- Lefort, F., and K.K.A. Roubelakis-Angelakis. 2001. Genetic comparison of Greek cultivars of *Vitis vinifera* L. by nuclear microsatellite profiling. Am. J. Enol. Vitic. 52:101-108.
- Lodhi, M.A., G.N. Ye, N.F. Weeden, and B.I. Reisch. 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol. Biol. Rep. 12:6-13.
- Lopes, M.S., M. Rodrigues dos Santos, J.E. Eiras Dias, D. Mendonça, and A. da Câmara Machado. 2006. Discrimination of Portuguese grapevines based on microsatellite markers. J. Biotech. 127:34-44.
- Lopes, M.S., K.M. Sefc, E. Eiras Dias, H. Steinkellner, M. Laimer Câmara Machado, and A. Câmara Machado. 1999. The use of microsatellites for germplasm management in a Portuguese grapevine collection. Theor. Appl. Genet. 99:733-739.
- Maletic, E., K.M. Sefc, H. Steinkellner, J.K. Kontic, and I. Pejic. 1999. Genetic characterization of Croatian grapevine cultivars and detection of synonymous cultivars in neighboring regions. Vitis 38:79-83.
- Martín, J.P., J.L. Santiago, O. Pinto-Carnide, F. Leal, M.C. Martínez, and J.M. Ortiz. 2006. Determination of relationships among autochthonous grapevine varieties (*Vitis vinifera* L.) in the northwest of the Iranian peninsula by using microsatellite markers. Genet. Resour. Crop Evol. 53:1255-1261.
- Martínez, L.E., P.F. Cavagnaro, R.W. Masuelli, and M. Zúñiga. 2006. SSR-based assessment of genetic diversity in South American *Vitis vinifera* varieties. Plant Sci. 170:1036-1044.
- Mello, L.M.R. 2007. Vitivinicultura brasileira: Panorama 2007. Embrapa Uva e Vinho. Empresa Brasileira de Pesquisa Agropecuária, Bento Gonçalves, Brazil.
- Meredith, C.P., J.E. Bowers, S. Riaz, V. Handly, E.B. Bandman, and G.S. Dangl. 1999. The identity and parentage of the variety known in California as Petite Sirah. Am. J. Enol. Vitic. 50:236-242.
- Moncada, X., F. Pelsy, D. Merdinoglu, and P. Hinrichsen. 2006. Genetic diversity and geographical dispersal in grapevine clones revealed by microsatellite markers. Genome 49:1459-1472.

- Narváez, C., M.H. Castro, J. Valenzuela, and P. Hinrichsen. 2001. Patrones genéticos de los cultivares de vides de vinificación más comúnmente usados em Chile basados en marcadores de microsatélites. Agric. Técnica 61:249-261.
- Núñez, Y., J. Fresno, V. Torres, F. Ponz, and F.J. Gallego. 2004. Practical use of microsatellite markers to manage *Vitis vinifera* germplasm: Molecular identification of grapevine samples collected blindly in D.O. El Bierzo (Spain). J. Hortic. Sci. Biotech. 79:437-440.
- Pommer, C.V., C.P. Ferri, F.P. Martins, I.R.S. Passos, M.M. Terra, and E.J.P. Pires. 2000. Agronomic and phenological characterization of grape genotypes kept in collection at Jundiaí, Brazil. Acta Hortic. 523:211-219.
- Riaz, S., K.E. Garrison, G.S. Dangl, J.M. Boursiquot, and C.P. Meredith. 2002. Genetic divergence and chimerism within ancient asexually propagated winegrape cultivars. J. Amer. Soc. Hort. Sci. 127:508-514.
- Sánchez-Ecribano, E.M., J.P. Martín, J. Carreño, and J.L. Cenis. 1999. Use of sequence-tagged microsatellite site markers for characterizing table grape cultivars. Genome 42:87-93.
- Sefc, K.M., F. Regner, J. Glössl, and H. Steinkellner. 1998. Genotyping of grapevine and rootstock cultivars using microsatellite markers. Vitis 37:15-20.
- Sefc, K.M., F. Regner, E. Turetschek, J. Glössl, and H. Steinkellner. 1999. Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome 42:367-373.
- This, P., et al. 2004. Development of a standard set of microsatelitte reference alleles for identification of grape cultivars. Theor. Appl. Genet. 109:1448-1458.
- Thomas, M.R., and N.S. Scott. 1993. Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). Theor. Appl. Genet. 86:985-990.
- Yuste, J., J.P. Martín, J.A. Rubio, E. Hidalgo, P. Recio, J.C. Santana, C. Arranz, and J.M. Ortiz. 2006. Identification of autochthonous grapevine varieties in the germplasm collection at the ITA of Castilla y León in Zamadueñas Station, Valladolid, Spain. Spanish J. Agric. Res. 4:31-36.