Identity and parentage of some South American grapevine cultivars present in Argentina

G. ALIQUÓ[1], R. TORRES[1], T. LACOMBE[2], J.-M. BOURSIQUOT[2], V. LAUCOU[2], J. GUALPA[3], M. FANZONE[1], S. SARI[1], J. PEREZ PEÑA[1] and J.A. PRIETO[1]

[1] Estación Experimental Agropecuaria Mendoza (EEA), Instituto Nacional de Tecnología Agropecuaria (INTA), Luján de Cuyo (5507), Mendoza, Argentina; [2] Montpellier SupAgro – Institut National de la Recherche Agronomique, Unité Mixte de Recherche Amélioration Génétique et Adaptation des Plantes (UMR AGAP), F-34060 Montpellier Cedex I, France; [3] Universidad Nacional de Río Cuarto (UNRC), Córdoba, Argentina

Corresponding author: Dr Jorge A. Prieto, email prieto.jorge@inta.gob.ar

Abstract

Background and Aims: Based on 19 nuclear simple sequence repeat markers and parental analysis, we aimed to identify and propose the pedigree of different accessions held at the Estación Experimental Agropecuaria Mendoza of the Instituto Nacional de Tecnología Agropecuaria germplasm collection. The results were compared with data recorded in large, international databases.

Methods and Results: We identified 37 different cultivars, of which 18 were original and not previously identified. The parentage analysis showed that European cultivars, such as Muscat of Alexandria, Muscat à Petits Grains, Listán Prieto, Mollar Cano and Malbec, were involved in natural crossings resulting in different South American cultivars.

Conclusions: Many of the cultivars identified here represent unique individuals based on their genotype. The number of cultivars that participated as progenitors in the origin of South American germplasm is higher than previously thought.

Significance of the Study: Germplasm collections planted many years ago play a key role in the conservation and characterisation of genotypes that otherwise may have been lost. It is highly probable that there might be other genotypes not identified and mixed in old vineyards. The identification, rescue and conservation of these genotypes are a challenge to preserve the existing genetic variability.

Keywords*: criollas, cultivar identification, microsatellite, parentage analysis*

Introduction

The history of the grapevine (*Vitis vinifera* L.) in America dates back to the 15th century, when it was first introduced to the Antilles during the Spanish colonisation (Maurín-Navarro 1967, Martínez et al. 2006). It was cultivated with success in Mexico a few years later and then in Peru in the early 16th century (Martínez et al. 2006, 2008). Subsequently, it expanded to the rest of the South American colonies (Milla-Tapia et al. 2013). There is some evidence that it was brought to America from the Canary Islands (Agüero et al. 2003), but it is still unclear if it was introduced in the form of seeds, cuttings, plants or buds (Martínez et al. 2008). During the first steps of South American viticulture and for more than 300 years, the Spanish cultivar Listán Prieto was the predominant cultivar (Lacoste et al. 2010). It was grown under different names, such as Criolla Chica or Criolla de Vino in Argentina, Uva País in Chile, Negra Corriente or Rosa del Perú in Peru, Misión in Mexico and Mission in USA (Agüero et al. 2003, Martínez et al. 2006). Besides Listán Prieto, Muscat of Alexandria was also a major cultivar in Argentina, where it was called Uva de Italia (Alcalde 1989). It was introduced from Spain to Mendoza by Jesuit missionaries in the early 18th century, and it was one of the most cultivated white cultivars until the end of the 20th century. Some recent studies have demonstrated that these two cultivars (Listán Prieto and Muscat of Alexandria) are the progenitors of the main South American cultivars, including the group of Torrontés, Criolla Grande Sanjuanina, Cereza and Pedro Giménez, for instance (Agüero et al. 2003, Martínez et al. 2006, Milla-Tapia et al. 2007, Durán et al. 2011, Lacombe et al. 2013, Boursiquot et al. 2014). The Peruvian cultivar Quebranta, which is a crossing between Listán Prieto and Mollar Cano (This et al. 2006), another Spanish cultivar, would be an exception to this group.

Molecular markers have proved to be a valuable tool to organise and manage germplasm collections, to identify genotypes and to study the parental relationships among different cultivars (Sefc et al. 2000, Dangl et al. 2001, Ibáñez et al. 2003, This et al. 2004). Among the different molecular markers, microsatellites have been widely used to study grapevine, including American cultivars (Agüero et al. 2003, Martínez et al. 2003, 2006, This et al. 2006, Milla-Tapia et al. 2007). These previous studies have contributed to describe and characterise the American germplasm in terms of genetic diversity and originality. As mentioned before, based on simple sequence repeat (SSR) analysis, these studies have provided the identity and origin of the main and more traditional cultivars and served as a complement to the American germplasm ampelographic studies (Storni 1927, Vega et al. 1962, Alcalde 1989, 2008).

Ancient and traditional vineyards consisted of a mixture of different grapevine cultivars, frequently derived from seeds (Agüero et al. 2003). The fact that vines were sexually propagated, together with the possibility of natural crossing among the different cultivars, may have caused a higher variability than expected, which has not been sufficiently

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explored. For more than 500 years, some of these genotypes have evolved and adapted to local conditions, where no phylloxera existed in the natural environment. Therefore, it is likely that the genetic diversity found in America may be a reservoir of new generated genotypes and also of ancient genotypes lost in Europe due to the later phylloxera crisis. Many of them no longer exist in commercial vineyards due to replants to adapt to international markets and consumer preferences. Despite this genetic erosion, it is most likely that some ancient genotypes still exist and are confined in germplasm collections (This et al. 2006), created before this varietal replacement. Thus, further research is required to determine accurately the extent and origin of this genetic diversity. Identifying and characterising the accessions conserved in the collections is necessary to determine the true number of cultivars, their relationships and the identification of unique genotypes in these collections (This et al. 2006). In this work, we used SSR markers to identify different accessions conserved at the Estación Experimental Agropecuaria (EEA), Mendoza Instituto Nacional de Tecnología Agropecuaria (INTA) germplasm collection. These accessions were collected in different regions of the country more than 50 years ago (Gonzalez and Vega 1949, Vega 1950, Alcalde 1972), and many of them appear to be no longer cultivated in commercial vineyards. Some of these accessions have been characterised only through ampelographic methods and were conserved in the collection until now. Finally, based on historical data, we also tested some hypotheses concerning the possible parental relationships between these criollas cultivars and some of those of Europe.

Materials and methods

Site and plant material

We studied 50 grapevine accessions maintained in the INTA Vine Collection, located at the INTA EEA Mendoza experimental campus (lat. 33°S; long. 68°51′W), Argentina. This collection is referenced with the code ARG01 in the *Vitis* International Variety Catalogue (http://www.vivc.de/). The vineyard was planted in 2008, with the vegetal material extracted from the germplasm collection originally planted in 1949 and replanted in 1979. The original collection was created through a prospection of Argentinean vineyards older than 100 years, located in the west and north-west regions of the country (Gonzalez and Vega 1949). Later, the size of the collection increased by other regional prospections and through exchange with European countries, when more than 500 cultivars from European collections were introduced (Alcalde 1972). The local genotypes that could not be identified through ampelography were conserved in the collection. Many of them were named as 'Criolla' followed by a number (e.g. Criolla No. 125). During the 1970s, a collection with more than 50 autochthonous cultivars existed (Vega 1976, 1977). Unfortunately, that collection was lost and only a few accessions were conserved.

The study included 13 accessions (Table 1) corresponding to the main local cultivars that have already been previously described (Agüero et al. 2003, Martínez et al. 2006, Milla-Tapia et al. 2007, Durán et al. 2011, Lacombe et al. 2013), 24 accessions corresponding to unreported cultivars that have

Table 1. Accession code and local name of the genotypes studied in the germplasm repository at Estación Experimental Agropecuaria (EEA), Instituto Nacional de Tecnología Agropecuaria (INTA), Mendoza, Argentina.

Non genotyped accessions		Accessions corresponding to local genotyped cultivars		Accessions corresponding to European cultivars	
Accession code	**Local name**	**Accession code**	**Local name**	**Accession code**	**Local name**
24–12	Albillo	39–09	Cereza	00–05	Cot 598
52–04	Blanca Oval	26–13	Criolla Blanca	08–13	Criolla Chica
30–09	Blanca Oval Cuyana	49–04	Criolla Grande	47–13	Criolla Chica Ballista
41–13	Canela	50–01	Criolla Grande	12–14	Criolla Centenaria del Perú
29–12	Canelón	31–15	Moscatel Amarillo	00–01	Criolla Chica Salteña
39–10	Canelón	15–10	Pedro Giménez	00–02	Criolla Chica Salteña
07–05	Canelón	07–07	Quebranta	00–19	Malbec (clon 19 INTA)
46–07	Cereza Elipsoidal	02–14	Quebranta del Perú	34–05	Mollar Negra
38–11	Cereza Italiana	18–02	Torrontés Mendocino	49–06	Muscat à Petits Grains
03–02	Criolla No. 1	16–15	Torrontés Riojano	15–01	Muscat de Frontignan
05–03	Criolla No.125	17–13	Torrontés Sanjuanino	28–05	Muscat of Alexandria
31–14	Criolla No. 6	24–13	Torrontés Sanjuanino	30–04	Muscat of Alexandria
08–05	Ferra	27–12	Uva Anis	48–11	Palomino
48–12	Ferral	–	–	–	–
50–14	Fintendo	–	–	–	–
26–14	Huevo de Gallo	–	–	–	–
28–14	Huevo de Gallo	–	–	–	–
00–03	Malvasia Criolla	–	–	–	–
28–12	Moscatel Blanco	–	–	–	–
29–11	Moscatel Rosado	–	–	–	–
36–04	Moscatel Rosado	–	–	–	–
04–03	Negra Mole	–	–	–	–
00–04	Torrontés Sanjuanino Glabro	–	–	–	–
52–02	Valency	–	–	–	–

not yet been described nor identified previously and 13 accessions presumed to correspond to European cultivars in order to test some hypotheses about the possible parentage of those local cultivars (Table 1). The accessions Criolla Chica Salteña (codes 00–01 and 00–02), Malvasía Criolla (00–03) and Torrontés Sanjuanino Glabro (00–04) have been incorporated recently into the collection. Criolla Chica Salteña was brought from the Cafayate Valley in Salta (north of Argentina), while Malvasía Criolla and Torrontés Sanjuanino Glabro were collected from a vineyard in Mendoza.

Molecular analysis

For each accession studied, young leaves were sampled and frozen at −80°C. The DNA was extracted according to Laucou et al. (2011), using a plant mini kit (DNeasy plant mini kit, Qiagen, Hilden, Germany). Two independent samples were analysed for each vine. A set of 19 microsatellites was selected to genotype the accessions, including the set of eight microsatellite markers previously chosen for the screening of grapevine cultivars by the European *Vitis* database (This et al. 2004), and the *Vitis* International Variety Catalogue (VIVC): VVS2 (Thomas and Scott 1993), VVMD5, VVMD7, VVMD27, VVMD28, VVMD32 (Bowers et al. 1996, 1999), VrZAG62, VrZAG79 (Sefc et al. 1999). Eleven additional markers previously used to assess grapevine diversity were also included: VVMD21 (Bowers et al. 1999), VrZAG67, VrZAG83, VrZAG112 (Sefc et al. 1999), and VVIp60, VVIp31, VVIn16, VVIh54, VVIb01, VMC1b11, VVIq52 (Merdinoglu et al. 2005, Laucou et al. 2011). Given the genetic proximity of the accessions studied, these additional markers allowed us to strengthen the parentage analysis (Lacombe et al. 2013).

A final volume of 10 µL containing 10 ng total DNA, polymerase chain reaction buffers 1X (Invitrogen, Carlsbad, CA, USA), $MgCl_2$ (2 mmol), dNTP's (0.16 mmol), direct primer [γ-33P] (0.25 µmol), reverse primer (0.25 µmol) and 0.15 U Platinum Taq DNA Polymerase (Invitrogen) was amplified (Mastercycler Gradient, Eppendorf, Hamburg, Germany). The PCR cycles were as follows: an initial denaturation step at 94°C for 7 min; 35 cycles of 45 s at 94°C, 45 s at specific annealing temperature, 90 s at 72°C; and final extension of 20 min at 72°C. The DNA amplification fragments were sized using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and the molecular size was obtained with Gene Mapper v.3.7 software (Applied Biosystems).

Data analysis

The SSR data were used to estimate different genetic parameters with the software CERVUS v3.0.7 (Kalinowski et al. 2007). The alleles number per locus (N_a), the range of allele size (bp), the frequency of null alleles (F_{na}), the expected (H_e) and observed heterozygosity (H_o), polymorphic information content (PIC) and average non-exclusion probability for identity of two siblings (NE-SI) were estimated.

The loci data from the core set of eight SSR proposed by the VIVC were used to estimate the differences in allele size between our dataset and reference data for the same loci recorded in the international catalogue, which contains genotypes from all over the world (Lacombe et al. 2013). After standardisation of SSR allele size, we identified the different accessions by comparing our microsatellite genotypes with the data contained in the VIVC database. In a second step, all the SSR profiles were compared with the Institut National de la Recherche Agronomique (INRA) database generated from the French reference collection of INRA Domaine de Vassal (Laucou et al. 2011), which comprises more than 2500 different accessions.

Once the identity of each accession was established, the relationship among genotypes and parentage analysis was established using the 19 SSR markers analysed. We compared the profile of each accession to verify synonyms and homonyms, and parentage analysis was performed with CERVUS v3.0.7 (Kalinowski et al. 2007). Parent assignment was based on loci matching and logarithm of the odds (LOD) scores comparing the 19 microsatellites data for each tested accession with its putative parents. The LOD score, which is the natural logarithm of the likelihood, was calculated separately for each candidate parent and for the three genotypes together. We allowed a maximum of two loci mismatching, and the parentage relationship was considered significant when the trio confidence was higher than 95% of probability.

Results and discussion

Microsatellite analysis

The analysis of 19 microsatellite loci showed a total of 109 alleles (Table 2). The allele size ranged from 78 (VVIq52) to 307 (VVIb01). Except for the locus VVIn16 and VVIp60, the observed heterozygosity (H_o) was higher than the expected heterozygosity (H_e) for all the microsatellites, which is often the case in diversity studies in grapevine (Sefc et al. 2000, Ibáñez et al. 2003, Milla-Tapia et al. 2007).

Accession identity/original genotypes

The 50 different *V. vinifera* accessions were grouped into 37 different genotypes after the analysis (Table 3). Data of all microsatellites analysed are presented in Tables S1 and S2. The comparison of the eight reference loci with the VIVC database revealed that 19 genotypes (nine European and ten local cultivars) corresponded to previously genotyped cultivars already recorded in the database, whereas the 18 remaining corresponded to unreported genotypes with no homology in the VIVC database nor in that of the INRA Domaine de Vassal. Because no homology was found with those databases, and based on historical data and morphological observation, it is highly probable that many of these unreported genotypes had a local origin. Another possibility, although less strong, is that they were introduced from Europe, where they were lost thereafter. Whether they are criollas or ancient missing European cultivars, it is important to highlight that some of these plants may be unique individuals of these cultivars, which were conserved and preserved from extinction in the INTA collection.

The 18 original genotypes found were as follows (Table 3, Figure 1): Blanca Oval, Canela, Canelón, accession 39–10 (accession name: Canelón), Cereza Elipsoidal, Criolla No. 1, Criolla No. 6, Criolla No. 125, Ferra, Fintendo, Huevo de Gallo, Malvasía Criolla, Moscatel Blanco, Moscatel Rosado, accession 30–04 (accession name: Muscat of Alexandria), Torrontés Sanjuanino Glabro, accession 15–01 (accession name: Muscat de Frontignan) and accession 50–01 (accession name: Criolla Grande). The accession Criolla No. 6 presents the same microsatellite profile of the Criolla No. 5 present in the Vassal collection. Our accession Canela (41–13) is a different cultivar to the Canela or Kanela reported in the VIVC catalogue, which has a Spanish origin.

Synonyms

Our results showed that many accessions were duplicates. As expected, the accessions named as Criolla Chica, Criolla Chica

Table 2. Genetics parameters obtained from the 19 microsatellite loci used in cultivar identification.

Locus	N_a	bp	F_{na}	H_o	H_e	PIC	NE-SI
VVS2	8	133–155	−0.083	0.875	0.752	0.706	0.404
VVMD5	8	224–242	−0.084	0.842	0.724	0.676	0.423
VVMD7	6	233–263	−0.001	0.650	0.641	0.592	0.478
VVMD27	6	180–195	−0.082	0.925	0.804	0.762	0.371
VVMD28	8	234–268	−0.032	0.800	0.751	0.703	0.405
VVMD32	9	240–274	−0.101	0.975	0.815	0.779	0.364
VrZAG62	7	186–204	−0.096	0.950	0.805	0.765	0.371
VrZAG79	7	243–259	−0.104	0.974	0.810	0.770	0.368
VVMD21	4	241–263	−0.137	0.975	0.752	0.694	0.408
VrZAG67	5	124–150	−0.108	0.846	0.715	0.664	0.429
VrZAG83	4	189–200	−0.146	0.875	0.670	0.593	0.465
VrZAG112	5	228–245	−0.034	0.725	0.699	0.635	0.443
VMC1b11	5	166–187	−0.142	0.800	0.635	0.565	0.487
VVIb01	4	288–307	−0.093	0.575	0.476	0.390	0.605
VVIh54	4	164–180	−0.188	0.675	0.490	0.415	0.592
VVIn16	3	148–153	0.012	0.350	0.378	0.341	0.670
VVIp60	4	317–325	0.014	0.500	0.516	0.403	0.582
VVIp31	8	176–193	−0.039	0.795	0.734	0.689	0.415
VVIq52	4	78–84	−0.108	0.825	0.675	0.596	0.462
Total	109	–	–	–	–	–	–

bp, Range of allele size; F_{na}, frequency of null alleles; H_e, expected heterozygosity; H_o, observed heterozygosity; N_a, number of alleles; NE-SI, average non-exclusion probability for identity of two siblings; PIC, estimated probability of identity.

Ballista, both Criolla Chica Salteña, and Criolla Centenaria del Perú corresponded to the Spanish variety Listán Prieto. Of all loci analysed, however, the two accessions named Criolla Chica Salteña were homozygous in the VVMD28 and VVIp60 loci. Even if these five accessions are genetically similar, they present some morphological differences related to leaf shape, bunch size, compactness, berry size and colour. Given that Listán Prieto has a long history of cultivation in America, from California to Argentina, these morphological differences may reflect the existence of clonal variants as it has been found in other cultivars such as Pinot Noir (Hocquigny et al. 2004). For instance, the accession 08–13 presents mainly male flowers with bunches containing few berries. This accession corresponds to the accession Criolla Chica No. 2 also present in the Vassal collection (see online database http://bioweb.supagro.inra.fr/collections_vigne). The accessions Quebranta and Quebranta del Perú, Mollar Negra and Negra Mole corresponded to Quebranta and Mollar Cano, respectively. The accessions Blanca Oval and Blanca Oval Cuyana were identical for 18 loci, with only one allele different in the VVMD32 marker (Table S1). Similarly, the accessions Cereza and Cereza Italiana were identical for 17 loci, with differences in one allele in the markers VrZAG79 and VVIb01, the loci being heterozygous in one accession and homozygous in the other. These two cultivars did not present any ampelographic differences, excepting that Cereza Italiana presents lighter berry coloration than Cereza.

Homonyms

Besides these synonyms, many homonyms were also found. The accessions 28–05 and 30–04 were present in the collection both as Muscat of Alexandria. Our analysis showed that while the accession 28–05 corresponds to this cultivar, the accession 30–04 is another genotype, which could not be identified. This genotype, even if it presents similar morphological and anatomical characteristics, showed differences for nine loci with Muscat of Alexandria (Tables S1, S2). This unknown cultivar has not been previously cited, and we could not find any correspondence with the reference collection of Domaine de Vassal (Laucou et al. 2011). In consequence, we cannot conclude if this cultivar has a local origin or whether it is a European cultivar that was lost in Europe after its arrival to America. Our analysis indicates that this cultivar shares with Muscat of Alexandria one allele in all loci analysed, excepting for the VVIp60. In consequence, the most probable hypothesis is that if VVIp60 is considered as a null allele, it may be a progeny of Muscat of Alexandria. Its origin, however, remains to be elucidated. Meanwhile we propose here to name this cultivar as Moscatel Pincanta. The word pincanta means 'brother' in Huarpe, the language of the Huarpe people, the native inhabitants of this region. A similar case was found with the accessions 49–06 and 15–01 recorded as Muscat à Petits Grains and Muscat de Frontignan (traditional synonym), respectively. Our results showed that the accession 49–06 was the true Muscat à Petits Grains while the accession 15–01 was an unreported cultivar. Here, we proposed to call it Moscatel Apicia, a name given by the ancient Romans to the Muscats because of the attraction that the berry flavour provokes on bees. Another case of homonymy corresponded to the accessions 49–04 and 50–01, recorded as Criolla Grande but were found to be different genotypes. While 49–04 is Criolla Grande Sanjuanina, the accession 50–01 is a different genotype not previously identified. This last accession presented smaller berries with more uniform and darker coloration. We proposed to name this cultivar Vega.

The three accessions named as Canelón (7–5, 29–12 and 39–10) were actually two different cultivars. Whereas the accessions 7–5 and 29–12 were two accessions of Canelón, the accession 39–10 presented differences in three loci (VVS2, VVMD28 and VVMD32; Table S1). Even if they are not the same cultivars, they are two genotypes highly related, with the same morphological and anatomical characteristics.

Table 3. Main characteristics of each accession and comparison of the genetic profiles obtained with the analysis of eight microsatellite loci with the existing genetic profile in the *Vitis* International Variety Catalogue and the Institut National de la Recherche Agronomique, Domaine de Vassal database.

Accession name	Accession ID	Berry colour	Flavour	Use	Sex	Country	Cultivar name in Argentina	VIVC Prime name	VIVC ID
Albillo	24–12	White	None	WT	H	ESP	Albillo	Albillo Real	247
Blanca Oval	52–04	White	Muscat	W	H	ARG	Blanca Oval	np	np
Blanca Oval Cuyana	30–09	White	Muscat	W	H	ARG	Blanca Oval	np	np
Canela	41–13	Pink	Muscat	WT	F	ARG	Canela	np	np
Canelón	29–12	Black	Muscat	W	F	ARG	Canelón	Canelón	14386‡
Canelón	07–05	Black	Muscat	W	F	ARG	Canelón	Canelón	14386
Canelón	39–10	Black	Muscat	W	F	ARG	Canelón Grande†	np	np
Cereza	39–09	Pink	None	WT	H	ARG	Cereza	Cereza	2390
Cereza Elipsoidal	46–7	Pink	None	WT	H	ARG	Cereza Elipsoidal	Cereza Elipsoidal	40018‡
Cereza Italiana	38–11	Pink	None	WT	H	ARG	Cereza	Cereza	2390
Cot (clone 598)	00–05	Red	None	W	H	FRA	Malbec	Cot	2889
Criolla Blanca	26–13	White	None	W	H	ARG	Criolla Blanca	Criolla Blanca	40025‡
Criolla Chica Ballista	47–13	Red	None	W	H	ESP	Criolla Chica	Listán Prieto	6860
Criolla Chica	08–13	Red	None	W	M	ARG	Criolla Chica	Criolla Chica 2	17461
Criolla Chica Salteña	00–01	Red	None	W	H	ESP	Criolla Chica	Listán Prieto	6860
Criolla Chica Salteña	00–02	Red	None	W	H	ESP	Criolla Chica	Listán Prieto	6860
Criolla Centenaria del Perú	12–14	Red	None	W	H	ESP	Criolla Chica	Listán Prieto	6860
Criolla Grande	49–04	Red	None	WT	F	ARG	Criolla Grande	Criolla Grande Sanjuanina	3241
Criolla Grande	50–01	Red	None	WT	F	ARG	Vega†	np	np
Criolla No. 1	03–02	Red	None	W	H	ARG	Criolla No. 1	np	np
Criolla No. 6	31–14	Red	None	W	F	ARG	Criolla No. 6	np	np
Criolla No. 125	05–03	Red	None	W	F	ARG	Criolla No. 125	np	np
Ferra	08–05	Black	None	W	F	ARG	Ferra	np	np
Ferral	48–11	Black	None	T	H	ESP	Breval Negro	Breval Negro	24609
Fintendo	50–14	Black	None	T	H	ND	Fintendo†	np	np
Huevo de Gallo	26–14	White	Muscat	T	H	ND	Huevo de Gallo	np	np
Huevo de Gallo	28–14	White	Muscat	T	H	ND	Huevo de gallo	np	np
Malbec	00–19	Red	None	W	H	FRA	Malbec	Cot	2889
Malvasía Criolla	00–03	White	Muscat	W	H	ARG	Malvasía Criolla†	np	np
Mollar Negra	34–05	Red	None	WT	H	ESP	Mollar	Mollar Cano	7901
Moscatel Amarillo	31–15	White	Muscat	WT	H	ARG	Moscatel Amarillo	np	np
Moscatel Blanco	28–12	White	Muscat	WT	F	ARG	Moscatel Blanco	np	np
Muscat of Alexandria	28–05	White	Muscat	WT	H	GRE	Moscatel de Alejandría	Muscat of Alexandria	8241
Moscatel de Alexandria	30–04	White	Muscat	WT	H	ND	Moscatel Pincanta†	np	np
Moscatel Rosado	29–11	Pink	Muscat	WT	F	ND	Moscatel Rosado	Moscatel Rosado	8040‡
Moscatel Rosado	36–04	Pink	Muscat	WT	F	ND	Moscatel Rosado	Moscatel Rosado	8040‡
Muscat de frontignan	15–01	White	Muscat	WT	H	ND	Moscatel Apicia†	np	np
Muscat à Petits Grains	49–06	White	Muscat	WT	H	GRE	Moscato d'Asti	Muscat à Petits Grains Blancs	8193
Negra Mole	04–03	Red	None	WT	H	ESP	Mollar	Mollar Cano	7901
Palomino	48–11	White	None	WT	H	ESP	Palomino	Palomino Fino	8888
Pedro Giménez	15–10	White	None	W	H	ARG	Pedro Giménez	Pedro Giménez	24977
Quebranta	07–07	Pink	None	WT	H	PER	Quebranta	Quebranta	9840
Quebranta del Perú	02–14	Pink	None	WT	H	PER	Quebranta	Quebranta	9840
Torrontés mendocino	18–02	White	None	W	F	ARG	Torrontés Mendocino	Torrontés Mendocino	15161
Torrontés Riojano	16–15	White	Muscat	WT	H	ARG	Torrontés Riojano	Torrontés Riojano	15162
Torrontés Sanjuanino	24–13	White	Muscat	WT	H	ARG	Torrontés Sanjuanino	Torrontés Sanjuanino	17350
Torrontés Sanjuanino	17–13	White	Muscat	WT	H	ARG	Torrontés Sanjuanino	Torrontés Sanjuanino	17350
Torrontés Sanjuanino Glabro	01–04	White	Muscat	W	F	ARG	Torrontés Sanjuanino Glabro†	np	np
Uva Anís	27–12	White	Muscat	W	H	ARG	Uva anís	Uva anís	40830‡
Valeny	52–02	Pink	None	WT	H	ESP	Valency	Valenci Tinto	12865

†Cultivar name adopted here. ‡No simple sequence repeat data are available in the VIVC database for these cultivars. ARG, Argentina; ESP, Spain; F, female; FR, France; GRE, Greece; H, hermaphrodite; M, male; ND, non-determined; PER, Peru; T, table grape; VIVC, *Vitis* International Variety Catalogue; W, winegrape; np, cultivar not present in the VIVC.

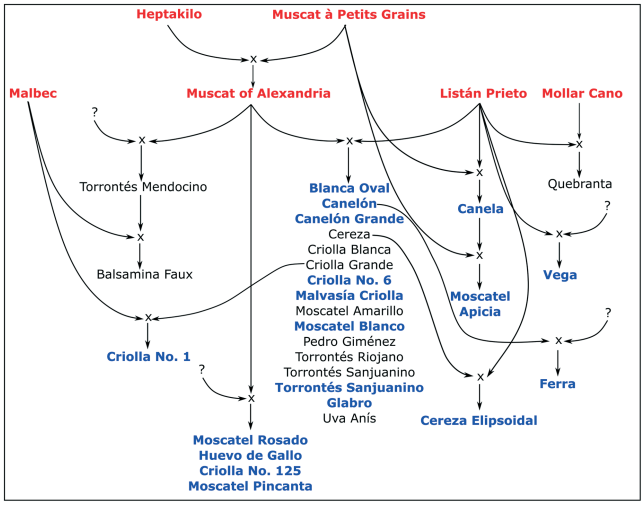

Figure 1. Schematic pedigree and parental relationship of criollas cultivars showing European cultivars (■), new cultivars determined in the present work (■) and cultivars with parentage already published (■) by Agüero et al. (2003), Boursiquot et al. (2009, 2014), Durán et al. (2011), Milla Tapia et al. (2007) and This et al. (2006).

Because of this similarity, they were probably introduced under the same name. Here, we propose to call the accession 39–10 Canelón Grande given its higher vigour and vegetative growth. Something similar occurred regarding the accessions Cot and Malbec (clone 19 INTA), which were different in two loci (VrZAG79 and VrZAG62) illustrating a clonal variation (Table S1). The difference observed in the VrZAG79 could be explained as a lag while reading the values obtained in the analysis, while the difference in the VrZAG62 corresponds to a classical mutation observed between clones (Pelsy 2010).

Parental determination

The parental analysis using 19 SSR was performed in order to obtain more information about the origin of some possible local cultivars, which may be unique, and to highlight the broad genetic diversity of the South American germplasm. This number of markers has been used in much of the literature undertaking parental analysis (Di Vecchi Staraz et al. 2007, Ibáñez et al. 2009, Laucou et al. 2011, De Andrés et al. 2012, Lacombe et al. 2013), including the previous studies concerning South American cultivars (Agüero et al. 2003, Milla-Tapia et al. 2007, Durán et al. 2011). The LOD scores for the trios analysed (the two parents and the progeny) ranged between 8.05 and 18.5 (Table 4). These values are lower than those obtained in some studies (Zinelabidine et al. 2012, Lacombe et al. 2013,) but are in the same range as those obtained by others (El Oualkadi et al. 2011, Stajner et al. 2015). The values obtained here for cultivars whose pedigree is already known (e.g. Cereza, Criolla Grande Sanjuanina, Pedro Giménez and Torrontés Riojano) are similar to the LOD scores presented by Lacombe et al. (2013). So, considering that the pedigrees of some of these cultivars have been published previously (Agüero et al. 2003, Milla-Tapia et al. 2007, Lacombe et al. 2013), and based on historical data (Vega 1950, 1977) and ampelographic traits (Alcalde 1989), we conclude that our LOD scores allow us to validate parental relationships within this group of criollas cultivars.

The analysis performed in order to establish the parents of each cultivar was based on previous findings proposing that the main South American cultivars originated from several natural crossings between Listán Prieto and Muscat of Alexandria (Agüero et al. 2003, Milla-Tapia et al. 2007). In accordance with these authors, our results confirmed that 15 local cultivars derived mainly from these crossings (Table 4, Figure 1). As previously reported, among this group, we confirmed Torrontés Riojano, Torrontés Sanjuanino, Moscatel Amarillo (Agüero et al. 2003), Criolla Grande Sanjuanina, Cereza (This et al. 2006, Milla-Tapia et al. 2007), Pedro Giménez (Durán et al. 2011), and Uva Anís and Criolla Blanca (Lacombe et al. 2013). Our results allowed the addition of Blanca Oval, Canelón, Canelón Grande, Criolla No. 6, Moscatel Blanco, Malvasia Criolla and Torrontés Sanjuanino Glabro to this list. We also confirmed that Quebranta originated from a cross between Mollar Cano and Listán Prieto (This et al. 2006). We could also confirm that Torrontés Mendocino is a progeny of Muscat of Alexandria while the other parent still remains unknown (Agüero et al. 2003). The results also showed that Cereza Elipsoidal was a cross between Listán Prieto and Cereza (Table 4).

Interestingly, the results obtained here suggest that many other cultivars have contributed to the origins of some cultivars in natural crossings (Table 4, Figure 1). We found that Canela was derived from a crossing between Muscat à Petit Grains and Listán Prieto. In the same way, Moscatel Apicia was derived from a backcrossing between Muscat à Petit Grains and Canela. The fact that Muscat à Petits Grains participated in a crossing is not surprising because it was introduced into Peru by the Jesuit missionaries in the late 16th century. Then it was spread to the south arriving in Mendoza in the middle of the 17th century (Lacoste et al. 2010), where it surely was cultivated in the same plots mixed with other cultivars.

Surprisingly, the results showed that Criolla No. 1 comes from a crossing between Malbec and Criolla Grande Sanjuanina. Similar results were obtained by Boursiquot et al. (2014) who showed that Balsamina Faux (another Argentinian cultivar from Patagonia) was derived from a cross between Malbec and Torrontés Mendocino. These results confirm that the process of natural crossing continued over the years after the arrival of the first *V. vinifera* plants and that other cultivars, besides Listán Prieto, Muscat of Alexandria and Mollar Cano, played an important role in the genesis of South American genetic diversity. Here, we found that Malbec, Muscat à Petit Grains, Criolla Grande Sanjuanina and Cereza also participated in natural crossings. The arrival of Malbec and many other French cultivars to Argentina, and more particularly to Mendoza, dates to 1853 (Lacoste 2013). They were brought by the French agronomist Michel Pouget when Listán Prieto and Muscat of Alexandria were the main cultivated cultivars (Lacoste 2013). The historical evidence suggests that the different cultivars were cultivated with no phylloxera and mixed within the same plots, which could have favoured the natural crossing between cultivars. Malbec was widely accepted by growers, and later (around 1936), more than 70 000 ha were cultivated with Malbec in the country, more than today. The findings that Malbec participated in the crosses that originated Criolla No. 1 and Balsamina Faux open the possibility to the hypothesis that other French cultivars [e.g. Bonarda de Argentina (Corbeau), Pinot Noir, Cabernet Sauvignon, Merlot, Tannat and Syrah] brought also at that time could have contributed to the genesis of other criollas cultivars. This hypothesis is actually being tested by analysing at the INTA collection different genotypes that have never been identified, and they present some morphological and

Table 4. Putative parent–offspring relationships resulting from the logarithm of the odds (LOD) scores obtained in the parental analysis of 37 cultivars.

Offspring ID	Putative parent pair	Pair loci mismatching	Pair LOD score*	Trio loci mismatching	Trio LOD score*	Trio confidence
Canela	Listán Prieto	0	5.91	0	15.00	†
	Muscat à Petits Grains	0	6.68			
Canelón	Listán Prieto	0	10.72	0	8.74	†
	Muscat of Alexandria	0	9.54			
Canelón Grande	Listán Prieto	0	5.53	0	9.76	†
	Muscat of Alexandria	0	4.42			
Cereza	Listán Prieto	0	10.08	0	12.13	†
	Muscat of Alexandria	0	3.32			
Criolla Blanca	Listán Prieto	0	8.31	0	11.52	†
	Muscat of Alexandria	0	6.49			
Criolla Grande	Listán Prieto	0	8.14	1	16.06	†
	Muscat of Alexandria	1	7.75			
Criolla No. 6	Listán Prieto	0	3.47	1	9.00	†
	Muscat of Alexandria	1	2.93			
Fintendo	Breval Negro	0	16.82	nc	nc	†,**
	nf	nc	nc			
Moscatel Amarillo§	Listán Prieto	0	3.55	0	9.92	†
	Muscat of Alexandria	0	3.46			
Moscatel Apicia	Canela	0	6.59	2	15.69	†
	Muscat à Petits Grains	2	8.22			
Moscatel Blanco	Listán Prieto	0	2.04	0	12.8	†
	Muscat of Alexandria	0	2.95			
Pedro Giménez	Listán Prieto	0	2.69	1	10.47	†
	Muscat of. Alexandria	0	1.44			
Torrontés Sanjuanino Glabro	Listán Prieto	0	2.20	0	13.07	†
	Muscat of Alexandria	0	3.10			
Torrontés Riojano	Listán Prieto	0	2.12	0	10.86	†
	Muscat of Alexandria	0	1.48			
Uva Anís	Listán rieto	0	7.45	0	14.30	†
	Muscat of Alexandria	0	4.71			
Vega	Listán Prieto	0	3.84	nc	nc	†,**
	nf	nc	nc			
Torrontés Mendocino	Muscat of Alexandria	0	3.20	nc	nc	†,**
	nf	nc	nc			
Torrontés Sanjuanino	Listán Prieto	0	5.68	1	9.75	†
	Muscat of Alexandria	1	4.37			
Blanca Oval¶	Listán Prieto	0	1.49	0	8.05	†
	Muscat of Alexandria	0	4.36			
Cereza Elipsoidal	Cereza	0	7.34	1	11.91	†
	Listán Prieto	0	3.85			
Criolla No. 1	Criolla Grande	0	3.65	1	14.97	†
	Malbec	1	10.52			
Quebranta§	Listán Prieto	0	3.87	0	18.5	†
	Mollar Cano	0	10.41			
Huevo de Gallo	Muscat of Alexandria	0	1.09	nc	nc	†,**
	nf	nc	nc			
Moscatel Rosado	Muscat of Alexandria	1	1.40	nc	nc	†,**
	nf	nc	nc			
Criolla No. 125	Muscat of Alexandria	1	1.89	nc	nc	†,**
	nf	nc	nc			
Ferra	Canelón	0	2.57	nc	nc	†,**
	nf	nc	nc			
Malvasia Criolla	Listán Prieto	0	1.47	0	8.65	†
	Muscat of Alexandria	0	1.06			

*LOD scores were calculated for two putative parents separately (Pair LOD score) and both together including the offspring (Trio LOD score). A maximum of only two loci mismatching was allowed, and the parentage relationship was considered significant when the trio confidence was higher than 95% of probability. †Significant at 95% of confidence. §The number of pair loci compared was 18. ¶The number of pair loci compared was 17. nf, the second putative parent for the cultivar was not found. nc, not calculated. **When the second putative parent was not found, the significance refers only to the pair LOD score.

anatomical characteristics similar to criollas. It also leads to the idea that these genotypes may not be as old as other local cultivars (e.g. Criolla Grande Sanjuanina, Cereza and Torrontés Riojano). The analysis of some traits related to berry size and composition confirms the relationship between Criolla No. 1 and Malbec. Some analysis performed (not presented) showed that berry composition in terms of the concentration of phenolic substances and berry size is similar. Some recent studies show that Malbec wines and berries have a high concentration of dihydroquercetin-3-glucoside (around 500 μg/g of skin), a flavonoid compound (Fanzone 2012). This compound has not been found in other cultivars such as Cabernet Sauvignon or Merlot, suggesting that it could be a specific trait for this cultivar. Our analysis showed that Criolla No. 1 presented a moderate concentration (48 μg/g of skin) of dihydroquercetin-3-glucoside whereas other criollas cultivars do not (e.g. Canela, Cereza and Vega). Furthermore, analysis of the berries of the cultivars Prunelard and Magdeleine Noire de Charentes, two ancient cultivars, which are the progenitors of Malbec (Boursiquot et al. 2009), revealed that this compound was also present in both cultivars, but it was three times higher (170 μg/g of skin) in Magdeleine Noire de Charentes than in Prunelard (67 μg/g of skin). Apparently, Malbec inherited this trait related to berry and wine composition from these cultivars, and Criolla No. 1 from Malbec. Actually, the berry size, colour intensity, uniformity and wine composition of Criolla No. 1 are much closer to that of Malbec than to that of the wines coming from the other traditional criollas cultivars (e.g. Criolla Grande Sanjuanina and Cereza). Figure 1 illustrates the possible origin and parental relationship of the South American cultivars studied till now.

The origin of Criolla No. 125, Huevo de Gallo, Ferra, Fintendo, Moscatel Rosado, Mosatel Pincanta and Vega still remains unknown. Fintendo derives from Breval Negro, a Spanish cultivar, Ferra from Canelón, and Vega from Listán Prieto, but we could not find the other parent for these cultivars. Also, we found that one putative parent of Moscatel Rosado, Moscatel Pincanta, Huevo de Gallo and Criolla No. 125 may be Muscat of Alexandria (Table 4). Our Moscatel Rosado is different to that conserved in the Domaine de Vassal repository, which comes also from Argentina but is a tablegrape. It is also different to the Moscatel Rosado present in Portugal (code VIVC 24147), which is a cross between Mantuo and Muscat of Alexandria (http://www.vivc.de/). In South America, Moscatel Rosado is a traditional cultivar, known in Peru as Rosada del Perú, in Chile as Moscatel Rosada Pastilla or Moscatel Rosada de Talca. It has been cultivated for a long time as a tablegrape and for winemaking. In Chile for instance, it is one of the authorised cultivars by the Pisco industry. It presents some similarities on its morphological characteristics with the cultivar Moscatel Gordo Morado, an ancient and probably extinct cultivar mentioned by Roxas Clemente y Rubio (1807). It may have been introduced to Peru, around the late 17th century (Lacoste et al. 2010) and then expanded to the south, arrived in Chile and then in Argentina (Storni 1927). Alcalde (1989), however, classified it as a criolla cultivar, probably originating from a seed and having lost the relationship with the European parents.

Conclusions

This study reveals that the diversity of South American cultivars may be higher than previously thought. The constant vineyard transformation, new plantings of internationally accepted cultivars replacing ancient local ones, may have led to important genetic erosion all over the region. Germplasm collections planted many years ago play a key role in the conservation and characterisation of genotypes that otherwise may have been lost. We consider that the results obtained in this study indicate that there may be other genotypes not identified and mixed in old vineyards. The case of Malvasia Criolla, recently found in a vineyard mixed with Torrontés plants, is an example of the diversity that might still be conserved. The case of Criolla No. 1 and Balsamina Faux also illustrates the continuous generation of local cultivars after the arrival of the Spaniards. The rescue and conservation of these genotypes is necessary in order to preserve this genetic variability. Furthermore, the study and knowledge of their oenological and agronomical characteristics or in terms of tolerance to biotic or abiotic factors may open new avenues for research in the future and for new breeding programs.

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Supporting information

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Table S1. Genetic profiles of 37 varieties and two clonal variant of Cot at 8 microsatellite loci. Allele sizes are given in base pairs.

Table S2. Genetic profiles of 37 cultivars and two clonal variants of Cot at 11 microsatellite loci. Allele sizes are given in base pairs.