Vitis **61**, 47–51 (2022) DOI: 10.5073/vitis.2022.61.47-51

Prospection and identification of traditional-heritage Peruvian grapevine cultivars (*Vitis vinifera* L.) from Ica and Cañete valleys

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Summary

Interest in ancient and autochthonous cultivars has increased in recent years since they are directly related to the historical and cultural values of a region. Ica and Cañete valleys (Peru) have a long history of grapevine cultivation and the aim of this study was to identify phenotypes corresponding to the most used varieties for the local production of Pisco and wine. The 29 samples were collected in 17 vineyards in the Ica and Cañete valleys, and were analyzed using 20 molecular markers and 5 morphological descriptors according to the OIV. Results showed that the 29 collected samples corresponded to 11 genotypes: seven traditional cultivars and four unknown genotypes not registered previously. The known cultivars were 'Muscat of Alexandria', 'Listán Prieto', 'Quebranta', 'Moscatel Rosado', 'Pedro Giménez', 'Muscat Hamburg' and 'Palomino Fino'. The four not registered genotypes are locally known as 'Mollar de Ica', 'Moscatel Rosada de Cañete', 'Prieta Mollar' and 'Torontel'. All of them correspond to offspring of traditional-heritage Peruvian cultivars. We also found a phenotypic variation of 'Listán Prieto' with muscat flavor and identified phenotypic berry color variations in 'Quebranta'. This study increases the knowledge of traditional Peruvian grape varieties and highlight the genetic variability preserved in the traditional vineyards of local producers.

K e y w o r d s : cultivar identification; 'Listán Prieto'; 'Muscat of Alexandria'; parentage; traditional varieties.

Introduction

The interest in ancient and autochthonous grapevine cultivars has increased in recent years in the world and, therefore, their correct identification has become necessary in order to link them with their historical background and cultural value. Within the genetic resource of each region, several synonyms and homonyms remain to be clarified which, together with the existence of unidentified and unnamed cultivars, make it difficult to assess the value of a given cultivar in a region (Martin *et al.* 2011).

The recognized wine territory of Ica and Cañete valleys have a long history, having been closely related to the Viceroyalty of Perú (current city of Lima) that in 1540 was the first colonial center of viticulture in South America (Vázquez de Espinosa 1969, Toro-Lira 2018). The Spanish colonizers and missionaries introduced *Vitis vinifera* L., between the end of the 15th century and the beginning of the 16th century from the Iberian Peninsula and the Canary Islands (De la Vega 1609, Cobo 1890, Mena García 2011). According to historical references, the varieties were 'Listán Prieto', 'Muscat of Alexandria', 'Muscat à Petits grains', 'Mollar Cano', 'Albillo Real', 'Breval Negro', 'Palomino Fino' and 'Moscatel Rosado' (D'Ornellas 1863, Storni 1927, Husdon 1867). These introduced cultivars were the basis for the development of viticulture in the region and gave origin to autochthonous cultivars resulting from crossings between them (Milla Tapia *et al.* 2007). In South America, these crossings are commonly known as "Criollas", being traditional cultivars of the region. In Peru, 'Quebranta' has been cited as a descendant of 'Mollar Cano' and 'Listán Prieto' (This *et al.* 2006, Aliquó *et al.* 2017). Given that ancient vineyards still exist where no varietal changes have been performed, the prospection of these vineyards could lead to non-identified varieties that could be employed as source for Pisco elaboration. Some uncertainty about the identity and the origin of these unknown genotypes still remain. Similar studies have been carried out in other South American countries (Lacombe *et al*. 2013, Aliquó *et al.* 2017, Mejía *et al.* 2020, Milla Tapia *et al.* 2007) and have contributed positively to the development of the industry. The traditional vineyards of Ica and Cañete present several varieties cultivated within the same plot which are used to produce varietal Pisco. The aim of the present study was to identify through molecular markers different phenotypes

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collected in ancient vineyards of Ica and Cañete valleys, presumed as traditional and autochthonous genetic resource. These minor varieties are grown mixed in the vineyards and are used for wine and Pisco production.

Material and Methods

According to vineyard prospection, 29 phenotypes from traditional vineyards located in Ica and Cañete valleys (Pisco Denomination of Origin, Peru) were selected (Tab. 1). The selected phenotypes were found in blocks used for the production of Pisco and in all cases were observed in more than one vineyard. Samples were genotyped with 20 nSSR (nuclear Simple Sequence Repeats): VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG79, VVMD21, VrZAG67, VrZAG83, VrZAG112, VVIp60, VVIp31, VVIn16, VVIh54, VVIb01, VMC1b11, VVIq52. DNA isolation, PCR conditions and alleles sizing were performed as previously reported (Aliqué *et al.* 2017). The identity and parental analysis were performed with Cervus 3.0 software (http://www.fieldgenetics.com/pages/home.jsp), including 120 genotypes (INTA Vine Collection, SSR base date, Argentina) as putative parents the 8 traditional varieties introduced in the colonial period (D'Ornellas 1863, Storni 1927, Husdon 1867) and their Criollas offspring (Agüero *et al*. 2003, Lacombe *et al*. 2013, Aliquo *et al*. 2017). Parent assignment was based on loci matching and logarithm of the odds (LOD) scores comparing the 20 nSSR data for each tested profiles with the putative parents. The LOD score, which is the natural logarithm of the likelihood, was calculated separately for each candidate parent and for the trio. We allowed a maximum of one locus mismatch (Lacombe *et al.* 2013). Parentage relationship was significant when the trio confidence was higher than 95 % probability.

We also performed a brief morphological description recording the flower sex (OIV 151), berry length (OIV 220), shape (OIV 223), flavor (OIV 236) and skin color (OIV 225). At least 10 observations per single trait were performed and the character status was assigned according to the OIV criteria (Muñoz *et al*. 2011).

Results and Discussion

We prospected 17 vineyards located in the Ica and Cañete valleys and 29 phenotypes were sampled corresponding to the most spread and used varieties for the local production of wine and Pisco. From all samples collected, our microsatellite analysis revealed 11 genotypes, 7 traditional cultivars and 4 unknown genotypes not registered in the international database VIVC (Tab. 1).

Variety identification: Our analysis allowed to identify 7 known cultivars: 'Muscat of Alexandria', 'Listán Prieto', 'Quebranta', 'Moscatel Rosado', 'Pedro Giménez', 'Muscat Hamburg' and 'Palomino Fino'. Several samples corresponded to 'Listán Prieto' and 'Muscat of Alexandria' (Tab. 1). Two types of phenotypes were recognized for 'Listán Prieto' in relation to berry characteristics. The true-to-type with spheroid berries, medium to small size, reddish-black color, soft pulp and neutral flavor (Alcalde 1989). The true-to-type 'Listán Prieto' samples were named as 'Negra criolla' (NC-1, NC-2) or 'Negra corriente' (NC-5) the most spread names used in Perú, and 'Prieta' (NC-4) as a local name used in the Cañete Valley (De La Cruz 1949). The other type of 'Listán Prieto' corresponded to samples MN-1 and MN-3, and were found with local names of 'Moscatel' and 'Moscatel Negro' respectively. Surprisingly, these samples presented berries with muscat flavor when ripe, and were smaller in size and lighter in color, closer to red-brownish (Fig. S1). The first reference to this phenotype was made by D'Ornellas (1863) referring as: black, with somewhat long and dense clusters, medium-sized berries, black and some whitish, sweet pulp and a little rough on the palate. Thereafter, it was cited by Vega (1966) as 'Moscatel Negro', due to its similarity with a black malvasia because of its muscat flavor. In 1970, an accession of this phenotype was introduced to the Vassal Collection (France), coming from vineyards of Ica valley, through the EEA Mendoza INTA Grapevine Collection (Argentina), with the name 'Moscatel Negro du Perou' according to its provenance (Boursiquot *et al.* 2014).

Several samples corresponded to 'Muscat of Alexandria' (I-1, I-3, I-6, I-7) commonly known in Peru as 'Italia' or 'Italia Dorada', whereas the varieties 'Moscatel Rosado' and 'Muscat Hamburg' are commonly known as 'Italia Rosada' (I-4) and 'Italia Negra' (IN-1) respectively. 'Moscatel Rosado', is an ancient variety present in Argentina and Chile, classified by Alcalde (1989) as Criolla, in connection with the founding varieties of South American viticulture. Its identification in Ica allows us to hypothesize that it may have been present in the vineyards of the Viceroyalty and then dispersed to the south, together with the other white muscat varieties. On the other hand, 'Muscat Hamburg' was introduced at the end of the 19th century as table grape and for winemaking. This variety is not related to the founding varieties, but its cultivation is associated with the other accessions known as 'Italia' (Hidalgo 2011).

The samples 'Quebranta Negra' (Q-6, Q-7), 'Quebranta Rosada' (Q-8), 'Quebranta Mollar' (Q-2) and 'Quebranta Blanca' (Q-9) corresponded to the genotype of 'Quebranta' (code *V*IVC 9840). Interestingly, these accessions differed in berry size and bunch color, with berries ranging from dark purple to pink and white. Coloration is not homogeneous between berries within a bunch and within bunches of the same plant. 'Quebranta negra' and 'Quebranata rosada' have darker and more homogeneous clusters, while those of 'Quebranta mollar' and 'Queranta blanca' are lighter variegated (Fig. S2), as already described by Vega (1966) and Hidalgo (2011). Differences in anthocyanin accumulation give rise to variations in berry color, however, hypotheses regarding the effect of possible clonal variations, genotype-environment interactions and plant health could be tested to explain the causes of these phenotypes (Vega *et al.* 2011, Torregosa *et al.* 2011, Martínez Zapater *et al.* 2014). We also identified 'Pedro Giménez' (I-5) mixed in an ancient vineyard of 'Muscat of Alexandria', that was unperceived by the vine grower.

Table 1

Identity analysis and morphological description of each variety identified. The genetic profiles were obtained with the analysis of 20 nuclear microsatellites markers and compared to the *V*IVC database. Local name, sample ID code and wine region of collection of Ica and Cañete are indicated

ID Sample	Wine region of collection	Local name	Prime name	*V*IVC code	Loci typed/ Matching/ Mismatching	pID	Status	Berry lenght	Berry shape	Berry skin color	Flowers sex	Taste	Country of origin
A-1	Santiago	Albilla	Palomino Fino	8888	20/20/00	1.95E-18	Exact match	short	globose	blanc	hermaphrodite	none	Spain
A-2	Guadalupe	Albilla											
A-3	Quilmaná	Albilla											
I-1	Quilmaná	Italia	Muscat of Alexandria	8241	20/20/00	2.84E-18	Exact match	long	obovoid	blanc	hermaphrodite	muscat	Greece
I-3	Santiago	Italia Dorada											
I-6	San Vicente	Italia											
I-7	San Vicente	Italia											
I-4	Santiago	Italia Rosada	Moscatel Rosado	8040	20/20/00	1.27E-17	Exact match	medium	obovoid	rose	female	muscat	--
I-5	Santiago	-	Pedro Giménez	24977	20/20/00	1.69E-15	Exact match	medium short	globose	blanc	hermaphrodite	none	Argentina
IN-1	Santiago	Italia Negra	Muscat Hamburg	8226	19/19/00	8.70E-24	Exact match	long	ovoid	noir	hermaphrodite	muscat	United Kingdom
NC-1	Santiago	Negra Criolla	Listán Prieto	6860	20/20/00	2.63E-15	Exact match	short medium	globose	noir	hermaphrodite	none	Spain
NC-2	Quilmaná	Negra Criolla											
NC-4	Pacarán	Prieta											
NC-5	Santiago	Negra Corriente											
MN-1	Santiago	Moscatel						short	globose	rouge	hermaphrodite	muscat	Peru
MN-3	Lunahuaná	Moscatel Negro											
Q-2	Lunahuaná	Quebranta Mollar	Quebranta	9840	20/20/00	1.22E-15	Exact match	medium	broad elliptic	noir rouge rose blanc	hermaphrodite	none	Peru
Q-4	Nuevo Imperial	Quebranta Rosada											
Q-6	Lunahuaná	Quebranta Negra											
Q-7	Nuevo Imperial	Quebranta Negra											
Q-8	Guadalupe	Quebranta Rosada											
Q-9	Nuevo Imperial	Quebranta Blanca											
M-5	Guadalupe	Mollar de Ica	--	--			Not match	medium	broad elliptic	rose	hermaphrodite	none	Peru
MR-1	Zuñiga	Moscatel Rosada de Cañete	--	--			Not match	medium	globose	rose	hermaphrodite	muscat	Peru
RC- 1	Nuevo Imperial												
NM- 1	Lunahuaná	Prieta Mollar	--	--			Not match	long	globose	rouge	hermaphrodite	none	Peru
T-1	Santiago	Torontel Peruano	--	--			Not match	medium	globose	blanc	hermaphrodite	muscat	Peru
T-2	Santiago												
T-3	Ocucaje												

* The wine region of Lunahuaná, Nuevo Imperial, Pacarán, Quilmaná, San Vicente and Zuñiga are located in Cañete and Guadalupe, Ocucaje and Santiago are located in Ica. pID: the probability that a single unrelated individual has the same genotype when the two genotypes match exactly.

Cultivated blocks with mixed varieties, where different genotypes with similar phenotypes coexisted for many years, have contributed to conserve traditional minority varieties. Finally, 'Albilla', mentioned by Roxas Clemente (1807) as 'Albilla de Lucena' and described in Peru by D'Ornellas (1863), was identified as 'Palomino Fino'. It could have been introduced by the Spanish colonizers for winemaking during the XV and XIV centuries.

Parental relations: The four unknown genotypes were compared with 45 profiles of Criollas varieties, the available genotype information obtained from the INTA Vine Collection (Argentina) and also compared with the *V*IVC data base, the profiles were not associated with registered varieties in any cases. They have distinctive morphological characteristics that allow them to be recognized in the vineyards, mainly in relation to ripe clusters and berry flavor (Fig. S3, Tab. 1). To deepen the knowledge about their origin we performed parental analysis and it showed that they were offspring of traditional South American varieties (Tab. 2, Tab. S1). The samples collected as 'Torontel' (T-1, T-2, T-3), descend from the crossbreeding between 'Listán Prieto' and 'Muscat of Alexandria', as most of the *Criolla* varieties of South America (Milla Tapia *et al.* 2007). This variety is a full sibling of other 27 cultivars, including 'Torrontés Riojano', 'Criolla Grande Sanjuanina', 'Pedro Giménez' and 'Cereza', among others (Aliquo *et al.* 2017, Torres *et al.* 2021). 'Torontel' is one of the eight varieties authorized for Pisco production (INDECOPI 2011) and has a long tradition of cultivation. Three varieties with the same name have also been reported in Chile, one of them is registered in the *V*IVC (N°15465) but without SSR information (Milla Tapia *et al.* 2007; Ibacache *et al.* 2015). In addition, the information available is from only 6 SSRs (out of 9 selected by the *V*IVC) and is not calibrated in relation to the *V*IVC data. Therefore, it is not possible to perform Identity analysis comparisons. The identification made by Ibacache (2005) describes the phenotype of the variety and some differences in bunch and berry size were found. To avoid misidentifications, we propose to call the genotype described here as 'Torontel Peruano' maintaining this denomination given its current importance in the Pisco industry.

The 'Moscatel Rosada de Cañete' (MR-1, RC-1) is an offspring of 'Listán Prieto' and 'Moscatel Rosado', with a phenotype very similar to its muscat parent, being reported in Cañete valley since the beginning of the 20th century as a variety used for winemaking and distilling (De La Cruz 1949). On the other hand, 'Mollar de Ica' (M-5) and 'Prieta Mollar' (NM-1) are backcrosses of 'Quebranta' by their parents, 'Mollar Cano' and 'Listán Prieto', respectively. Backcrosses have been cited as a recurrent phenomenon in *criollas* (Aliquo *et al.* 2017, Torres *et al.* 2021). In Peru, the term "Mollar" is associated with varieties that present berries of different colors (red, pink and white) within the same bunch, with similar characteristics to 'Mollar Cano' (*versicolor* character, Roxas Clemente 1807), probably due to the fact that this variety is part of its genealogy. Grapevine was introduced to Peru almost 500 years ago, as well as in the rest of South America. The crosses between the varieties introduced at the beginning of South American viticulture are illustrative of the evolution in the local vineyards. This genetic resource was growing due to the emergent new varieties that were conserved, poorly identified, and cultivated in a minority form in traditional vineyards. The crosses and backcrosses are evidence of a complex system in which natural crosses and selection were present, as in other regions of the world (Maraš *et al*. 2020, Raimondi *et al*. 2020). These native progenies, with strong cultural roots and adaptation to the local environment, represent an opportunity for the development of local products, as in Ica and Cañete valleys. In addition, traditional and heritage varieties are being studied worldwide as a promising adaptation tool to climate change as they might be able to cope with expected future environmental conditions (Florez-Sarasa *et al*. 2020).

Conclusion

The use of molecular markers and morphological descriptors allowed us to highlight the existing diversity in the traditional-heritage Peruvian vineyard. Our results confirm the genetic relationships with cultivars from the Iberian Peninsula and demonstrate the close parentage relationships among *criollas* varieties of South America. We found 4 new varieties and confirmed 7 already known genotypes. Among the traditional varieties, we identified 'Moscatel Negro', a stable phenotypic variation of 'Listán Prieto', with double differences in color and muscat taste. In addition, local names were clarified and related to the prime names of the varieties. These contributions increase the knowledge about the traditional and autochthons varieties

Table 2

Putative parent-offspring relationships resulting from the logarithm of the odds (LOD) scores obtained in the parental analysis of 4 cultivars and 20 nSSR

ID Sample	Progeny (local name)	Parent 1	Parent 2	nSSR Contrib/ Mismatch	Trio LOD
M-5	Mollar de Ica	Mollar Cano	Quebranta	20/0	30.4
MR-1	Moscatel Rosada de Cañete	Moscatel Rosado	Listan Prieto	20/1*	41.9
NM- 1	Prieta Mollar	Listán Prieto	Quebranta	20/0	16.9
T-01	Torontel Peruano	Muscat of Alexandria	Listán Prieto	20/0	15.7

*The mismatch occurs when the Iq52 loci is homozygous in Moscatel Rosada de Cañete genotype.

of Ica and Cañete valleys allowing their use in winemaking and Pisco industry with distinctive aromatic characteristics. The plant resources of the present paper are conserved at the Ampelographic Grapevine Collection, EEA Chincha INIA (Instituto Nacional de Innovación Agraria, Perú). Generating policies to prospect, identify, conserve and characterize the local viticultural resources is crucial in order to strengthen the cultural identity of Peruvian winemaking industry.

Acknowledgements

This research was funded by projects Convenio de Colaboración Interinstitucional (co-funded by UNALM, Thalassa Taller de vino S. L. and Finca la Jaca S. A.), Perú and Proyecto Estructural I125 of the Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina. We thank the grape growers of Ica and Cañete for their collaboration in vineyard prospecting.

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Received October 10, 2021
Accepted March 2, 2022