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Molecular and ampelographic characterization of genotypes used for Pajarete wine, an old Denomination of Origin from Huasco and Elqui Valleys in northern Chile

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Summary

The characterization of 30 grape accessions used to produce Pajarete wine is presented, one of the first American "Denomination of Origin" (D.O.). Accessions were collected from small vineyards from the Huasco Valley in the Atacama Region, northern Chile, which are very heterogeneous for varietal assortment, as was demonstrated by microsatellite marker analysis. Eighteen different genotypes were identified, including the two varieties considered the founders of American viticulture, 'Listán Prieto' (syn. 'País') and 'Muscat of Alexandria'. A series of criolla varieties was also identified, some of them already described plus others not yet included in international databases. Based on nine SSR markers, allelic matchings suggest that four of these new criolla varieties (NN-311, NN-313, NN-368 and NN-370) derives from 'Listán Prieto' x 'Muscat of Alexandria', but three (NN-314, NN-276 and NN-369) are derived from 'M. of Alexandria' crossed with a yet undetermined parent. Seven criolla accessions were described by ampelography, including some not yet recorded in the European *Vitis* International Variety Catalogue (VIVC) or in the Instituto de Investigaciones Agropecuarias (INIA) database. Three accessions were considered as not appropriate for the production of Pajarete wine since two of them are table grape varieties and the other is the American variety 'Isabella' (*Vitis labrusca* x *V. vinifera*), all considered of poor quality for winemaking. All these findings are very useful to direct the conservation of this unique germplasm, as well as for small producers of Pajarete wine, since based on this information on the overall variety assortment of the area, they can now make informed decisions to improve their vineyard management and wine production.

Key words: *Vitis vinifera* L.; criolla varieties; Atacama; microsatellites; SSR; ampelography.

Introduction

Pajarete wine, as well as other wines and spirits from Chile such as "Asoleado de Cauquenes" and "Pisco", is a "Denomination of Origin" (D.O.) established as early as 1954 according to the Chilean "Law of Alcoholic Beverages" Nº 11,256, modified in 1969 (Chilean Law No. 17,105) and again in 1979 (Decreto No. 2,753) (Errázuriz-Tortorelli 2010). This D.O. rescues the tradition and antiquity of Pajarete production in the Huasco and Elqui Valleys in arid northern Chile, with a first record dated August 25, 1790; Terrise registered the first label for Pajarete wine in 1934 (reviewed in Castro *et al.* 2016). According to recent studies, Pajarete (also called "vino escogido") would be the oldest wine denomination in America (Castro *et al*. 2016). Today, there is still a reduced group of about 25 small grape growers and wine producers (Asociación de Productores de Pajarete del Valle del Huasco) who do their best to preserve this old and characteristic wine tradition.

However, the mixing of various grape varieties often not identified and the dominance of the "European paradigm" limited Pajarete wine distribution and restricted its commercialization to locals. In fact, by the end of the XIX century, the wines produced with French varieties became the most popular around the world, including Chile, where they were reinforced by local well-reputed specialists (Rojas 1950, Ibid 1987, Menadier 2012) who supported the French style of wines, disparaging the traditional production (reviewed in Castro *et al.* 2016).

In spite of this, Pajarete wine has overcome these difficulties and still remains today a genuine, generous red or white wine, locally produced and bottled from hand-picked grapes at their maximal maturity. In the production of Pajarete wine, harvested bunches are usually dehydrated by sun exposition with the aim of concentrating their components, including sugar, and increasing the alcohol level during fermentation (Ramírez and Ramírez 2015). Twisting bunch peduncle is also used, forcing the dehydration of the berries

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for days or weeks while still on the plant, thus raising the sugar to the desired level (Gil and Pszczólkowski 2015).

Since 2010, thanks to the improvement of vinification techniques and storage conditions while preserving the identity of the product, Pajarete has had a renaissance that has allowed a modest but significant level of exportation, primarily to other regions in Chile but also sporadically to international markets (Ramírez and Ramírez 2015). One of the critical aspects of this wine is the varietal palette used in its production. History tells us that grapevine cuttings arrived in America together with wheat and olive trees, comprising a set of fundamental Spanish biocultural elements. From the successful acclimation of vines in New Spain (current Mexico), these varieties began to spread throughout the continent, finally arriving in Chile through the Viceroyalty of Peru. The documentary records surveyed between the years 1550 and 1850 indicate that the first varieties introduced by the Spaniards were 'Listán Prieto' -the hegemonic variety in Chile for three centuries, where it was called 'País'- and 'Moscatel de Alejandría', along with 'Mollar' (Lacoste *et al.* 2015). From the coexistence and crossing of these varieties, new varieties called 'criollas' emerged in South America (Milla Tapia *et al.* 2007).

Pajarete producers (Olmedo-Valderrama 2007), make frequent reference to old varieties and the criolla ones derived from them. A special mention is given to 'País' and 'Uva de Italia' (syn. 'Muscat of Alexandria'), as well as the criolla varieties 'Moscatel de Austria' (syn. 'Torrontés Sanjuanino') and 'Uva Pastilla' (syn. 'Moscatel Rosada' or 'Rosada Pastilla'). Finally, 'Ardilla' is mentioned, probably in reference to 'Albilla,' another (probably) criolla variety scarcely planted, as well as 'Torontel' (syn. 'Torrontés Riojano') (Sotomayor 1987). Currently, some of these varieties are being rediscovered for their oenological and heritage value as key figures in beverages such as Pajarete, under study here (Duhart and Corona 2010, Pszczólkowski and Lacoste 2016).

The varieties currently used to produce Pajarete are unknown, but this situation is not new. In fact, neither the original law nor any of its modifications specified what varieties were allowed to be used, although table grapes were explicitly excluded. To some extent, it is assumed that the same varieties used for Pisco are used in Pajarete wine production (Castro *et al.* 2016). Other criolla varieties are presumably included in Pajarete musts. These criolla genotypes have been found in different locations in the northern agricultural valleys established along river canyons and oases in the Atacama Desert (Milla-Tapia *et al.* 2013). Several criolla varieties have been identified in the Andean region by analyzing their SSR pattern. Preliminary characterization of nine criolla varieties from Argentina and 16 accessions from Peru was carried out using SSR markers (Martínez *et al.* 2006). More recently, 18 new criolla varieties were described in Argentina, including their pedigree (Aliquó *et al.* 2017). It would not be surprising if some not yet characterized criolla varieties could be found in these old vineyards, where small farmers maintain their minute parcels, usually knowing every vine and its history of at-home propagation. These vines are in many cases unhealthy, referring to their virus sanitary status, with a reduced yield that results in low productivity and wine volume. This situation worsens due to a lack of rational, productive management for years, producing a non-stable offer to the market.

Identifying and tracing Pajarete wine genotypes could lead to the controlled propagation of vines to support and preserve this traditional dry wine. Vineyard management could be improved from this starting point, and a legal framework could be established that includes the definition of the varieties to be used from now on, which would be instrumental in preserving this traditional wine and helping to improve the standardization of its production.

Ampelography is the branch of botany concerning the identification and classification of grape varieties, including European *Vitis vinifera*, non-European *Vitis* species and inter-specific *Vitis* hybrids. This identification is based on morphological traits that include the description of the whole plant as well as the size, shape, color and hairs of leaves, shoots, rachis, bunches and berries, with different levels of detail (Fregoni 2005). There are also other ways in which varieties can be differentiated, including their chemical and biochemical composition (for example, secondary metabolites or isoenzyme profiles; Jahnke *et al.* 2012), but the most prevalent and successful approach is the so-called molecular ampelography, which is based on the comparison of alleles in specific loci of hypervariable regions of the genome, known as microsatellites or simple sequence repeats (SSRs) (Narváez *et al.* 2001, This *et al.* 2004, 2006). The best way to identify a variety or to describe a new one at present is by combining ampelography with SSR patterns, although other marker types such as Amplified Fragment Length Polymorphisms (AFLP) have been successfully applied to the study of criolla grapes (Martínez *et al.* 2003, Milla-Tapia *et al.* 2007). The main purpose of this study was to determine the genetic composition of the Pajarete varieties using ampelography and SSR patterns to provide this local and traditional industry with basic tools to support their production and reinforce their singularity. To some extent this is also a rescue program, as has been implemented in the Holy Land in recent years (Drori *et al.* 2017), where a combination of genetic profile and ampelographic study helped to distinguish feral and cultivated vines. We also present the genetic and ampelographic profiles for some genotypes not previously described, to guide the protection, preservation and local use of these valuable genetic resources.

This study of the diversity of grape varieties for Pajarete wine is limited to the Huasco Valley, representing an example of such research, not aspiring to be an exhaustive analysis of the topic. We opted to characterize the germplasm of this area first, because its vineyards are quite vulnerable due to factors such as its proximity to the Atacama Desert and the change of land use to mining.

Material and Methods

Leaves and tendrils of grapevines were collected during the 2017-2018 season (from October 2017 to March 2018), in the middle of the vegetative growing period at phenological stages from fruit setting to veraison, from different farms in Huasco Valley, district of Alto del Carmen, along the

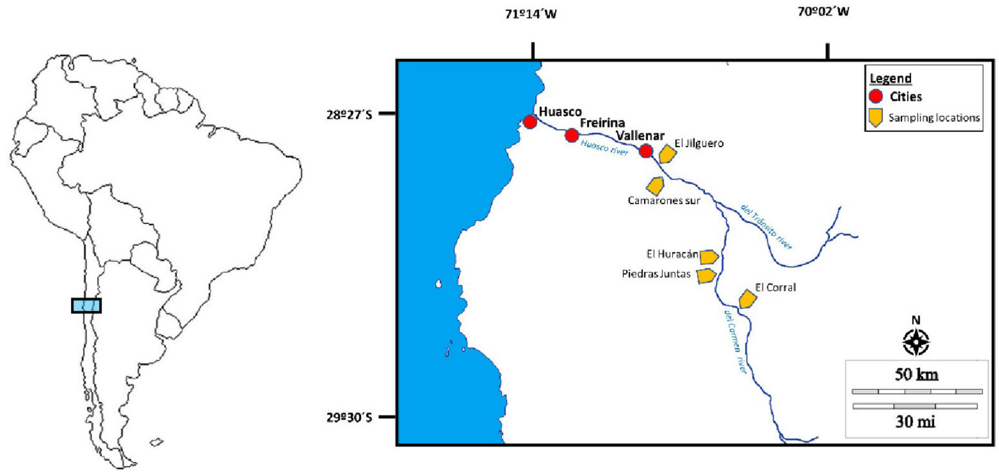

Figure: Map of the sampling locations in the Huasco Valley in Atacama Region, Chile.

valleys formed by the Tránsito and the Carmen Rivers that are tributaries of the Huasco River, in the Atacama Region of northern Chile (Figure).

Samples were collected from single vines that were labeled for future inspections, placed in labeled sterile plastic bags, refrigerated and transported to the laboratory for molecular analyses. Each organ was also photographed to aid the ampelographic characterization. Labeled vines were sampled and analyzed again later, to confirm their identity and collect cuttings to preserve the germplasm. The list of 30 samples is presented in Tab. 1 and suppl. material 1.

Molecular analyses were performed as described (Narváez *et al.* 2001, Milla-Tapia *et al.* 2007). For DNA extraction, ca. 100 mg of young leaves were ground in a buffered solution (CTAB, pH 8.0), nucleic acids were precipitated in ethanol with salts, and DNA was resuspended and stored in a mild buffered solution (TE 10 mM, pH 7.5). The amplification of SSRs was done with the six universally accepted markers proposed by This *et al.* (2004), expanded to nine SSRs as proposed by Ibáñez *et al*. (2009). Amplicons were separated under denaturing conditions in polyacrylamide gels and silver stained. Their size was determined by comparing the migration of alleles of well-characterized varieties ('Cabernet Sauvignon,' 'Chardonnay,' 'Muscat a petit grains blancs' and 'Pinot Noir'), as recommended by the *Vitis* International Variety Catalogue, *V*IVC (Maul *et al.* 2020). The results were compared to the *V*IVC database and the INIA database (manuscript in preparation), this latter containing abundant criolla genotypes. The parentage analysis was done using Cervus (v3.0.3) software (Kalinowski *et al.* 2007). A total of 18 individuals were considered for this analysis after sorting out clones from the initial 30 samples. Therefore, 16 offspring and 2 alleged parents namely 'País' ('Listán Prieto') and 'Muscat of Alexandria' were tested for parentage analysis considering the molecular data from the nine tested loci. The calculation of allele frequencies was done with the same software and a simulation of parentage analysis (offspring, 10,000; candidate parents, 2; proportion of loci sampled, 0.35; proportion of loci typed, 0.60; proportion of loci mistyped, 0.01; minimum typed loci, 2) was also considered in which parent pairs were tested assuming unknown sexes. For the ampelographic characterization, which focused on the genotypes with no records at *V*IVC, the descriptors and terminology used were those suggested by the International Organization of Vine and Wine (OIV, n.d.), following the protocol and evaluations proposed by Gil and Pszczólkowski (2015). This characterization was made at least in two seasons between 2017 and 2019. The phenological stages in which the ampelographic study was done were as follows: 1) When shoots were 0.5 to 1.0 m long, meaning after sprouting and flowering, which in the Huasco valley is during September; 2) shoots in active growth, with the presence of fully developed adult leaves, in October; 3) bunches reaching full maturity (18° to 22° Brix, depending on the genotype), but never over-ripened; this happens by February and March in this valley.

Results

Genotype characterization of Pajarete varieties: The first step for the characterization of the 30 accessions from Huasco Valley (suppl. material 1) was to determine their SSR allele pattern, presented in Tab.1. With this, it was possible to identify every genotype and compare them with available databases. We found 18 different genotypes, among which at least two pairs were quite similar, but distant enough to consider them as different varieties. Comparisons were made based on local names as well as on their allele patterns, first revising our own (INIA) database built from genotypes identified in Chilean vineyards, and then checking the matching with the *V*IVC database. The first group of accessions corresponded to the two varieties considered as the founder genotypes of American viticulture (Milla-Tapia *et al.* 2007), 'Listán Prieto' (five accessions; local synonyms 'País' and 'Negra') and 'Muscat of Alexandria' (five accessions; synonyms used here are 'Italia', 'Italia Antigua', 'Italia Mejorada' and 'Moscatel de Alejandría'; see Tab. 1). A third old Spanish variety found in this valley was 'Mollar Cano' (syn. 'Rosada Chaucha'); interestingly, this variety has been found very rarely in this region and to the South, being more common in Peruvian vineyards. It is well documented that 'País' has been present in Chile since the middle of XVI century, brought by the first Spanish conquerors (Milla-Tapia *et al*. 2007), while 'Muscat of Alexandria' was introduced at some point in the

Table 1

SSR allelic patterns (genotype) for accessions collected in Huasco Valley, Atacama Region, and their matching with the *V*IVC reference catalogue (prime names), the INIA database, and the probability of being derived from the crossing of 'Listán Prieto' and 'Muscat of Alexandria'

Local name	Prime name *V*IVC catalogue	*V*IVC code	INIA code	LOD LPxMA*	SSR pattern VVMD-5	VVMD7	VVMD27	VVMD25	VVMD28	VVMD32	VrZAG62	VrZAG79	VVS2
Italia Mejorada (Camarones sur)	Pedro Giménez	24977	9	1,296	230-230	249-239	195-186	249-241	268-268	264-258	196-186	251-247	133-133
Italia Antigua (Camarones sur)	Flame Seedless	4141	56		238-236	253-239	186-182	249-241	244-244	272-250	188-188	251-247	151-133
Rosada	ND	ND	314	1,745	230-230	251-239	195-182	255-249	268-268	272-264	204-188	247-247	137-133
Huasquina	Huevo de Gallo	25796	124		234-230	249-239	195-190	249-239	268-258	264-256	196-186	259-247	149-135
Uva Frutilla	Isabella	5560	62		240-240	249-235	184-180	249-241	236-226	272-248	204-202	247-237	151-123
País (Camarones sur)	Listán Prieto	6860	183		242-230	249-239	190-186	241-239	244-244	258-256	196-194	251-243	135-133
Negra 1	ND	ND	370	5,657	234-230	249-249	195-186	249-239	244-244	264-256	194-186	251-247	135-133
Negra 2	Listán Prieto	6860	183		242-230	249-239	190-186	241-239	244-244	258-256	196-194	251-243	135-133
Italia Antigua (El Huracán)	Muscat of Alexandria	8241	93		234-230	251-249	195-180	249-249	268-244	272-264	204-186	255-247	149-133
Moscatel Amarilla	Pedro Giménez	24977	9	1,296	230-230	249-239	195-186	249-241	268-268	264-258	196-186	251-247	133-133
Uva Blanca	ND	ND	369		236-234	251-239	195-182	249-241	244-244	272-272	204-188	247-247	149-145
Negra Alargada	ND	ND	313	7,465	242-230	251-239	195-186	249-239	244-244	264-256	204-196	251-247	135-133
País (El Jilguero)	Listán Prieto	6860	183		242-230	249-239	190-186	241-239	244-244	258-256	196-194	251-243	135-133
Cardenal de Grano Chico	Cardinal	2091	26		238-228	249-249	186-180	255-255	268-244	272-252	186-186	255-251	135-135
Cardenal de Grano Grande	Cardinal	2091	26		238-228	249-249	186-180	255-255	268-244	272-252	186-186	255-251	135-135
Italia Antigua (El Jilguero)	Muscat of Alexandria	8241	93		234-230	251-249	195-180	249-249	268-244	272-264	204-186	255-247	149-133
Negra de Grano Redondo	Cereza	2390	315	9,255	234-230	251-249	190-180	249-239	244-244	272-258	204-194	255-243	149-135
Italia Mejorada (El Corral)	Muscat of Alexandria	8241	93		234-230	251-249	195-180	249-249	268-244	272-264	204-186	255-247	149-133
Moscatel de Austria	Torrontés Sanjuanino	17350	94	3,343	242-230	249-239	186-180	249-241	268-268	272-256	196-186	255-251	149-133
Pedro Giménez (San Francisco)	ND	ND	368	4,387	234-230	249-249	195-186	249-241	244-244	272-258	194-186	251-247	149-133
Italia Nativa	Muscat of Alexandria	8241	93		234-230	251-249	195-180	249-249	268-244	272-264	204-186	255-247	149-133
Albilla (El Corral)	ND	ND	368	4,387	234-230	249-249	195-186	249-241	244-244	272-258	194-186	251-247	149-133
Rosada Talquina	ND	ND	276		236-234	251-239	195-182	255-249	244-244	272-272	204-188	247-247	149-137
San Francisco	Black Prince	1405	232	5,824	234-230	249-249	195-190	249-241	244-244	272-258	194-186	247-243	133-133
País (El Corral)	Listán Prieto	6860	183		242-230	249-239	190-186	241-239	244-244	258-256	196-194	251-243	135-133
Italia Antigua	Muscat of Alexandria	8241	93		234-230	251-249	195-180	249-249	268-244	272-264	204-186	255-247	149-133
Albilla (Piedras Juntas)	ND	ND	311	7,180	230-230	249-249	195-190	249-239	268-244	264-258	194-186	247-243	149-135
San Francisco	Black Prince	1405	232	5,824	234-230	249-249	195-190	249-241	244-244	272-258	194-186	247-243	133-133
Rosada Chaucha	Mollar Cano	7901	312		242-224	239-239	182-182	255-241	258-244	272-252	196-188	259-247	145-143
País (Piedras Juntas)	Listán Prieto	6860	183		242-230	249-239	190-186	241-239	244-244	258-256	196-194	251-243	135-133

(*) The values indicate the logarithm of odds of having 'Listán Prieto' and 'Muscat Alexandria' as true parents.

following 200 years, probably through Mendoza, then part of Chile (Lacoste *et al*. 2015). A second group of genotypes corresponds to hybrids probably originating in America, and thus called "criolla" types; most of them derive from the cross 'Listán Prieto' x 'Muscat of Alexandria', but some are self-crosses of one of these varieties or crosses with unknown third genotypes (Aliquó *et al.* 2017). Some of these criolla types are also present in other countries in the Andean region (mostly Argentina) and have been described before, even registered in the *V*IVC catalogue. This group includes the varieties 'Cereza' (syn. 'Negra de grano redondo'), ´Huevo de Gallo´ (syn. 'Blanca ovoide' and 'Huasquina'), 'Torrontés Sanjuanino' (syn. 'Moscatel de Austria'), 'Pedro Giménez' (syn. 'San Francisco' and 'Moscatel Amarillo') and 'Black Prince' (syn. 'San Francisco'). These last two genotypes constitute a case of homonymy, a relatively common situation in old Chilean vineyards (unpubl. results). 'Pedro Giménez' (different from the Spanish variety 'Pedro Ximenes') is one of the most planted of these genotypes; interestingly, even when during the last two decades the planted area was reduced by 32 %, the current 10,000+ ha make it the first white wine variety in Argentina (Durán *et al*. 2011; www.argentina.gob.ar/inv). There was another group of seven criolla genotypes not found in the *V*IVC database; some of them have been also identified in other Chilean valleys in the south-central part of the country, where viticulture was predominant during the 17th to early 20th centuries (unpubl. results). As most of these genotypes do not have a registered name, they have been numbered according to the enrollment in the INIA database as "NN-numerical code". In this category were NN-311 ('Albilla'), NN-313 ('Negra Alargada'), NN-314 ('Rosada'), NN-368 ('Albilla'), NN-369 ('Uva Blanca') and NN-370 ('Negra-1'), plus NN-276 ('Rosada Talquina'), which is called 'Italiona' in the nearby Elqui Valley (Tab. 1); some of these latter have minor allele differences and so could be somaclonal variants, but at this point it is not possible to determine which are mutations and which originated from crossings. For instance, based on this nine SSR marker set, perfect allele matchings suggest that NN-311, NN-313, NN-368 and NN-370 derive from 'Listán Prieto' x 'Muscat of Alexandria', but NN-314, NN-276 and NN-369 would derive from 'M. of Alexandria' crossed with a still undetermined parent.

Finally, two genotypes corresponding to table grapes were also found, a result not totally unexpected because of the large number of vineyards currently involved in the production of grapes for fresh consumption in this region of Chile. These were 'Cardinal' (syn. 'Cardenal de grano chico' and 'Cardenal de grano grande') and 'Flame seedless' (syn. 'Italia antigua'). Also, a non-*vinifera* genotype that has been identified in southern vineyards, called 'Uva Frutilla', was found in this survey. This is *V. labrusca* x *V. vinifera* 'Isabella'.

Ampelographic characterization of newly identified grapevine accessions from Huasco Valley: Focusing on the new genotypes described only in Chile up to now, we decided to do a careful characterization and vis-à-vis comparison of the most similar accessions based on ampelographic studies (OIV descriptors). The following samples were analyzed and compared: a) Sample G6 ('Rosada Talquina'), corresponding to NN-276 (syn. 'Italiona'), *vs*. C3 ('Uva Blanca'), named NN-369; b) Sample A7, named NN-370 *vs*. G3 and G5, identified as NN-368 and known as 'Pedro Giménez' or 'San Francisco' (G3) and 'Albilla' (G5); and c) Samples T2 (also known locally as 'Albilla'), identified as NN-311, C4 ('Negra Alargada'), named NN-313, and A3 ('Rosada'), classified as NN-314.

Ampelographic description of accessions 'Rosada Talquina' (G6) and 'Uva Blanca' (C3), corresponding to NN-276 and NN-369: The sample 'Uva Blanca' (C3) exhibits an SSR pattern mostly similar to 'Rosada Talquina' (G6), except for two alleles at markers VVMD25 and VVS2. Since it has a different berry color and different alleles at two SSRs, these genotypes must be considered different varieties and not sports as was initially presumed (Tab. 2, suppl. material 2 and 3).

This is supported by the identification of some minor ampelographic differences in secondary traits for 'Uva Blanca' and 'Rosada Talquina', even though the primary ampelographic features are coincident.

Comparative description of accessions 'Pedro Giménez/San Francisco' (G3), and 'Albilla' (G5), both genotype 368: Accession 'Negra 1' (A7), identified as NN-370, a genotype resembling 'Oro Campo' (two out of 18 alleles different), should be compared to Pedro Giménez/San Francisco (G3) and Albilla (G5) by classical ampelography, since their SSR allelic patterns are the closest in this group. A description was made of the G3 and G5 accessions, which shared their genetic patterns (Tab. 2 and suppl. material 4 and 5). 'Negra 1' (A7; genotype NN-370), unfortunately, was not described by classical ampelography, except for the berry color, blue-black (as 'Oro Campo'). G3 and G5 accessions, known by growers as 'Pedro Giménez' or 'San Francisco' and 'Albilla' respectively, both with green-yellow berries, are nearly identical, as predicted from their non-differentiable SSR patterns, even though minor differences are observable in the classical ampelographic description. Deciding if each of these genotypes are somaclonal variants of 'Oro Campo' or independently derived from the LPxMA cross would require the analysis of a larger set of SSR markers.

Description of 'Albilla' (T2), 'Negra Alargada' (A3), and 'Rosada' (C4) accessions, the NN-311, NN-313 and NN-314 genotypes, respectively: The 'Albilla' (T2) accession, enrolled as NN-311 in the INIA database, 'Negra Alargada' (C4), named NN-313 and 'Rosada' (A3), named NN-314, were also described by classical ampelography (OIV, n.d.) (Tab. 2 and suppl. material 6 and 7). They present different genotypes and their classical ampelographic descriptions (OIV, n.d) also present large differences in main characteristics, among them berry color, green-yellow in 'Albilla' (T2), red in 'Negra Alargada' (C4) and pink in 'Rosada' (A3). They are consequently different varieties, not previously registered.

Table 2

Ampelographic description of accessions G6 ('Rosada Talquina'), C3 ('Uva Blanca'), G3 ('Pedro Giménez' - 'San Francisco'), G5 ('Albillla', 'El Corral), T2 ('Albilla', 'Piedras Juntas'), C4 ('Negra Alargada') and A3 ('Rosada')

Code OIV		Rosada Talquina	Uva Blanca	Pedro Giménez – San Francisco	Albilla (El Corral)	Albilla (Piedras Juntas)	Negra Alargada	Rosada
Young shoot								
OIV 001	Opening of the shoot tip	5	5	5	5	5	5	5
Shoot								
OIV 007	Color of the dorsal side of internodes	1	1	2	1	1	1	1
OIV 008	Color of the ventral side of internodes	2	3	2	2	2	3	1
OIV 009	Color of the dorsal side of nodes	1	1	2	2	1	1	1
OIV 010	Color of the ventral side of nodes	2	2	2	2	1	1	1
OIV 011	Density of erect hairs on nodes	1	1	1	1	1	1	1
OIV 012	Density of erect hairs on internodes	1	1	1	1	1	1	1
OIV 013	Density of prostrate hairs on nodes	1	1	1	1	1	1	1
OIV 014	Density of prostrate hairs on internodes	1	1	1	1	1	1	1
OIV 015-1	Distribution of antho-cyanin coloration on the bud scales	4	2	4	3	1	4	3
OIV 015-2	Intensity of antho-cyanin coloration on the bud scales	5	3	7	3	1	3	3
OIV 017	Length of tendrils	1	3	1	1	1	5	3
Mature leaf								
OIV 065	Size of blade	5	7	5	5	7	5	5
OIV 067	Shape of blade	3	3	3	3	3	3	3
OIV 068	Number of lobes	3	3	3	3	3	3	3
OIV 069	Color of the upper side of blade	5	5	5	5	5	7	5
OIV 070	Area of anthocyanin coloration of main veins on upper side of blade	2	2	2	2	2	1	1
OIV 073	Undulation of blade between main or lateral veins	1	1	1	9	1	1	NA
OIV 074	Profile of blade in cross section	5	5	2	5	1	2	NA
OIV 076	Shape of teeth	3	3	3	3	3	3	3
OIV 079	Degree of opening/ overlapping of petiole sinus	3	3	3	3	5	7	3
OIV 080	Shape of base of petiole sinus	1	1	1	1	1	1	1
OIV 081-2	Petiole sinus base limited by vein	1	1	1	1	1	1	1
OIV 082	Degree of opening/ overlapping of upper lateral sinuses	4	3	3	3	3	3	3
OIV 084	Density of prostrate hairs between main veins on lower side of blade	NA	NA	NA	NA	7	3	1
OIV 085	Density of erect hairs between main veins on lower side of blade	NA	NA	NA	NA	5	1	1

Tab. 2, continued

Code OIV		Rosada Talquina	Uva Blanca	Pedro Giménez – San Francisco	Albilla (El Corral)	Albilla (Piedras Juntas)	Negra Alargada	Rosada
OIV 089	Erect hairs on main veins on upper side of blade	1	9	NA	NA	NA	NA	NA
OIV 094	Depth of upper lateral sinuses	5	5	5	5	5	5	5
Bunch								
OIV 202	Length	7	7	NA	NA	9	7	7
OIV 204	Density	NA	NA	7	7	1	NA	NA
OIV 208	Shape	NA	NA	2	2	1	NA	3
Berry								
OIV 223	Shape	4	4	2	2	2	3	4
OIV 225	Color of skin	2	1	1	1	1	3	2

(*) NA, Information not available.

Discussion and Conclusions

In this study, the ampelographic and SSR analyses of Pajarete vines revealed several cultivars coexisting in Huasco Valley; most correspond to the two founding genotypes of the American viticulture, 'Listán Prieto' and 'Muscat of Alexandria' and criolla hybrids derived from them, plus the old Spanish variety 'Mollar Cano', quite common in Peru. A dendrogram illustrating its genetic diversity and relatedness is included (suppl. material 8). Some of these criolla hybrids are also present in other regions/countries, such as 'Cereza' and 'Torrontés Sanjuanino,' commonly found in Argentina, and 'Huasquina' (prime name 'Huevo de Gallo'). Other criolla genotypes have been described before in Chilean vineyards (Milla-Tapia *et al.* 2007) or are new descriptions, a remarkable situation of Pajarete germplasm. In this last group, we found the cultivars 'Albilla' (NN-311), 'Negra Alargada' (NN-313), 'Rosada' (NN-314), 'Rosada Talquina' (NN-276), also known as 'Italiona' in other valleys from northern Chile, and 'Uva Blanca' (NN-369), another criolla quite similar in the allele pattern, but differentiable at two loci. Together with NN-368 and NN-370 (rose and white genotypes), all these accessions constitute a wealth for the Pajarete producers. In many cases, variables of color have been described for widely planted varieties such as 'Pinot' and even 'Cabernet Sauvignon' (Walker *et al*. 2006). Color variants are not uncommon in other isolated places, such as the Canary Islands (Marsal *et al.* 2019). Their identification permits the initiation of viticultural and enological evaluation studies searching for varieties that could provide quality and personality to the generous wines for this D.O.; based on the evidence provided here, they could become recognized in the corresponding legislation.

This study will also allow excluding "contaminant genotypes", including table grape varieties which should be excluded from the D.O. Pajarete. Among them, 'Cardinal' and 'Flame Seedless' are well known, widely planted in the region. The most unexpected genotype corresponds to 'Isabella' (syn. 'Uva Frutilla'), a *Vitis labrusca* L. variety, whose berries have a foxy aroma and taste ("fox grape"), used in other viticulture areas for table, juice, or wine. The Chilean legislation permits wine elaboration only from grapes of *Vitis vinifera* L., and consequently it cannot be used to produce any type of wine, including 'Pajarete'. It is difficult to understand how this genotype arrived to this region, but its presence has also been described in other Chilean old vineyards from the Central Valley (unpublished results).

The role of grape varieties is crucial for the product identity, both for wines and spirits. The strategy of wine production in the New World countries has commonly appealed to the relevance of varietal wines as the basis of their marketing in the world market (Pszczólkowski and Lacoste 2016). While Europeans emphasize their terroir and their thousand years of winemaking history, the New World industry has focused on affirming the relevance of grape varieties as the identity of its products (Pszczólkowski 2014). Pajarete can be positioned precisely in this category of products with a high added value, associated with a D.O., because its raw material reflects this authenticity and exclusivity provided by the criolla varieties, cultivated for centuries in this region that carry all the cultural heritage of Chile.

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