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Prospection and identification of grapevine varieties cultivated in north Portugal and northwest Spain

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Summary

A broad prospection of grapevine plants was carried out in northwest Spain and north of Portugal during the period from 2002 to 2009. It included zones located in Galicia as well as in the provinces of León, Zamora and Salamanca in Spain and the region delimited by the right side of the Douro river and the spanish border in Portugal. A total of 669 accessions were sampled, studied and identified, both by using the six microsatellite loci included in the OIV descriptors list, plus ampelographic characters. As a result of the study a total of 53 different grapevine varieties were identified. Most of them correspond to minor varieties that are present in the zone. In many cases the same variety was detected in both countries, although denominated with different names that are synonymous. The molecular markers that were analyzed are those included in the OIV 801 to 806 descriptors, and correspond to the following loci: VVS2, VVMD5, VVMD7, VVMD27, ssrVrZAG62 and ssrVrZAG79. Allele sizes of the studied varieties are recorded. Relationships among these varieties seem to be possible. Recommended priority names for the studied varieties both in Spain and in Portugal are listed, in order to avoid misidentifications and reduce the incidence of homonymies.

K e y w o r d s : endangered varieties, microsatellites, misidentifications, synonymies, *Vitis* germplasm.

Introduction

The northwest of the Iberian Peninsula, including regions from two countries, Spain and Portugal, has been object of grapevine cultivation for centuries. High quality wines are produced in this area, like the portuguese Porto wine or the spanish Albariño as well as several others. During the last decades, the origin denomination (D.O.) regions have resulted in a reduction of the number of cultivated varieties (Ortiz and Villaseca 2004), with the risk of extinction of ancient and minor varieties. For a long period of time exchange of varieties between both countries has occurred, and at the present different names are used for denomination of a given variety, causing the apparition of synonymies. Also homonymies, or the use of the same name for different varieties, are present. Both facts together with the existence of unnamed accessions are a source of misidentification of grapevine varieties in the region.

For grapevine varietal identification and detection of synonymies and homonymies, as previously established (Ortiz *et al.* 2004), there is recommended the observation of ampelographic characters, particularly those that are stable and objective, as well as the analysis of those microsatellite markers for which information in databases exist.

In the present work a broad prospection has been carried out during the period 2002 to 2009 in the northwest of the Iberian Peninsula, in order to detect unknown or endangered varieties and avoid their extinction. The study is mostly focused in old plantations, trying to identify and recover minor or neglected varieties. In all cases a molecular characterization by microsatellites together with an ampelographic description has been carried out in order to identify the studied varieties, which in many cases had local names, and were unknown in others.

Material and Methods

Prospection and sampling has been carried out at several locations in the northwest of Spain and north of Portugal. In total 669 accessions were gathered. In many cases either isolated plants or plants located at old vineyards areas were sampled.

Molecular characterization was made with descriptors OIV 801 to 806 (OIV 2009). DNA was extracted from fresh young leaves following the Doyle and Doyle (1990) method, modified by Torres *et al.* (1993). PCR Amplification was carried out by a protocol previously indicated (Martín *et al.* 2003). Microsatellite analysis of the six loci was accomplished by following the specifications for each of them: VVS2 (Thomas and Scott 1993), VVMD5 and VVMD7 (Bowers *et al.* 1996), VVMD27 (Bowers *et al.* 1999), ssrVrZAG62 and ssrVrZAG79 (Sefc *et al.* 1999). Comparison of the obtained allele sizes for identification was carried out with the available grapevine database obtained in our laboratory, http://www.sivvem.monbyte.com/sivvem.asp, completed with other databases published.

Ampelographic characterization was carried out with 30 OIV descriptors (OIV 2009), 17 observed or measured in mature leaves (OIV codes 067, 068, 070, 071, 076, 079, 080, 081-1, 081-2, 082, 083-1, 083-2, 084, 085, 086, 087

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and 091), 5 in bunches (OIV codes 202, 203, 204, 206 and 208), and 8 in berries (OIV codes 220, 221, 223, 225, 231, 236, 241 and 244). In all cases the varietal identification was confirmed by molecular plus ampelographic results.

Results and Discussion

Many of the sampled accessions were collected either as unidentified or with local names. As result of the molecular and ampelographic characterization the prospected plant material was identified.

A total of 53 different varieties were detected (Tab. 1). In 26 of the cases the variety was present in samples from Spain and from Portugal, although with different names in both countries. Tab. 1 indicates the prime names in each country, that are the names recommended for use in order to avoid misidentifications and homonymies. These names were chosen according to their wider use in each country and also trying to avoid homonymies that may lead to misnaming. In this Table are also listed the synonymies that were confirmed by molecular plus ampelographic characterization. Most of them were already cited in the literature. A high number of synonymies was detected, including some previously published that have been confirmed in the present work, as well as several others not mentioned before.

Most of the identified accessions correspond to minor or endangered varieties. From all the names listed in Tab. 1, more than 75 % were already cited by García de los Salmones (1914), which indicates that they are old varieties. Many of them decreased in importance after the outbreak of phylloxera and the starting of the origin denominations (D.O.), which markedly reduced the number of authorized varieties.

There is a group of eight portuguese varieties that probably were introduced into Spain in more recent times. These are 'Periquita', 'Sousao Galego' 'Bical' or 'Borrado das Moscas', 'Folgasão', cultivated in several regions of Spain as Cagarrizo; Cornifesto, the Gajo Arroba of the Arribes del Duero; Budelho, known in some minor areas of Spain as Pedro Ximénez Canario; Terrantez that is the Torrontés portugués; and Boal Cachudo, the Torrontés of Galicia.

Three foreign varieties which are also cultivated with importance in other European countries have been discovered: 'Traminer' or 'Savagnin Blanc', found in several places of Galicia; 'Chasselas Doré', rather extensively cultivated in several regions of northwest Spain under the name of 'Temprano Blanco'; and 'Ugni Blanc' or 'Trebbiano Toscano', a white variety, also present in many European countries, that has been detected as isolated plants in the zone. 'Aramon', a french variety with a lesser importance, which has spherical and rather large berries, was detected in vineyards at El Bierzo (León). According to previous information in our laboratory, the studied microsatellites of 'Aramon' are coincident with Amor-nao-me-deixes, a minor variety from Portugal. Further molecular plus ampelographic studies are needed in order to confirm or reject this possible synonymy. Six minor and endangered varieties have been found: 'Mandón' (Rubio *et al.* 2005), that is different from the 'Mandó' grown in Catalonia (Spain), the first one located in plantations of the Arribes del Duero region; 'Río Abaixo', 'Pan y Carne' and 'Negreda' (González-Andrés *et al.* 2007), found at different localities at El Bierzo; 'Verdejo Colorado' (Rubio *et al.* 2005), from the Arribes del Duero, and 'Verdejo Serrano', present in the region of Sierra de Francia (Arranz *et al.* 2008).

Most of the microsatellite profiles for the varieties in Tab. 1 have been previously published and confirmed in the present study, as indicated in that Table, although microsatellite ssrVrZAG47 instead of VVMD27 was analyzed, being a difference of 20 bases between both. Tab. 2 completes the information for the studied varieties in respect to descriptors OIV 801 to 806. Although six markers provide not enough information to induce genetic relationships, we analysed the existing coincidences in the obtained allele sizes for the studied varieties in order to establish possible common origins among varieties, which should be deeply studied with a larger number of genetic markers.

There is one pair of varieties that are coincident in all alleles except two, 'Bruñal' and 'Prieto Picudo Tinto' that differ in one allele of VVMD7 and other of ssrVrZAG62.

There are also 93 pairs of varieties that share at least one allele from each of the six microsatellite loci, what indicates the possibility of having one parent in common, as well as the probable existing relationships among the grapevine varieties of the region. Three varieties, 'Bruñal', 'Prieto Picudo Tinto' and 'Traminer' are the ones that are present in most of these mentioned pairs and thus may have contributed to the genesis of the northwestern Iberian Peninsula varieties: i) 'Bruñal' with 'Traminer', 'Allarén', 'Gajo Arroba', 'Mencía', 'Juan García', 'Periquita', 'Prieto Picudo', 'Tinta Jeromo', 'Torrontés de Galicia' and 'Ugni Blanc'; ii) 'Prieto Picudo' with 'Traminer', 'Allarén', 'Brancellao', 'Godello', 'Graciano', 'Mencía', 'Juan García', 'Periquita', 'Sousao Galego' and 'Verdejo'; and iii) 'Traminer' with 'Agudelo', 'Bruñal', 'Cagarrizo', 'Espadeiro', 'Godello', 'Merenzao', 'Juan García', 'Pan y Carne', 'Budelho', 'Prieto Picudo', 'Puesto Mayor' and 'Verdejo'.

There are three varieties, 'Tempranillo', 'Moscatel de Grano Menudo' and 'Verdejo Colorado' that have no variety with at least one common allele for each microsatellite locus. Two of them are very old: 'Tempranillo', already cited by 'Alonso de Herrera' (1513) under the name of 'Aragonés', and 'Moscatel de Grano Menudo', or 'Muscat à Petits Grains', classified as Proles Orientalis by Negrul (1938), that has been proposed as the origin of most muscat varieties (Bronner 2003). Hence, they are not autochthonous for the studied area and probably are less genetically related with the others. In the case of 'Verdejo Colorado', it is a very little known variety, grown in the Arribes del Duero, with red berries (Gallego and Cidón 2005), and no information about its possible origin is available. Moreover, in the case of 'Tempranillo', unique genotypes occur in loci VVMD5, VVMD7, VVMD27 and ssrVrZAG62, again probably related with its non autochthonous origin.

Tab. 3 summarizes the allele frequencies for each analysed microsatellite in all the 53 varieties of Tab. 1. The number of alleles oscillates from 7 to 10 with an average of

Table 1

Recommended prime names in Spain and in Portugal, and confirmed synonymies of the 53 identified grapevine varieties

Notes[1]	Prime names		Berry colour[2]	Synonymies[3]	SSR profiles
	Spain	Portugal			
X	Agudelo		B	Chenin Blanc (F)	Martín *et al*. 2006
X	Albariño	Alvarinho	B		Martín *et al*. 2006
X	Albillo		B	Albillo de Madrid (S)	Martín *et al*. 2003
X	Allarén		B		Martín *et al*. 2003
E	Aramon		N		This study (Tab. 2)
P		Bical	B	Borrado das Moscas (P)	Martín *et al*. 2006
X	Brancellao	Alvarelhao	N	Brancelho (P)	Martín *et al*. 2006
X	Brujidera		N	Crujidera (S)	Martín *et al*. 2003
X	Bruñal	Alfrocheiro Preto	N	Albarín Negro (S)	Martín *et al*. 2006
P		Budelho	B	Pedro Ximénez Canario (S)	Martín *et al*. 2003
P	Cagarrizo	Folgasão	B		This study (Tab. 2)
X	Caiño Blanco	Cainho de Moreira	B	Caiño Branco (P), Alvarinhao (P)	Martín *et al*. 2006
X	Caiño Tinto	Borraçal	N	Caiño (S), Tinta Femia (S)	Martín *et al*. 2006
X	Cañorroyo		B		Martín *et al*. 2003
X	Castellana Blanca		B		Martín *et al*. 2003
X	Doña Blanca	Síria	B	Malvasía Blanca (S), Malvasía Castellana (S), Dona Branca (P), Moza Fresca (S), Malvasía (S), Cigüente (S)	Martín *et al*. 2006
X	Espadeiro	Padeiro	N		Martín *et al*. 2006
P	Gajo Arroba	Cornifesto	N		Martín *et al*. 2006
X	Godello	Gouveio	B	Verdejo Blanco (S), Verdejo (S), Verdelho Branco (P), Verdelho (P), Bastardo Ruzo (P)	Martín *et al*. 2006
X	Graciano	Tinta Miúda	N		Martín *et al*. 2003
X	Juan García		N	Mouratón (S)	Martín *et al*. 2006
X	Lairén		B		Martín *et al*. 2003
X	Legiruela		B		Martín *et al*. 2003
X	Loureira	Loureiro	B	Loureiro Blanco (S), Marqués (S)	Martín *et al*. 2006
X	Macabeo		B	Viura (S)	Martín *et al*. 2003
X	Mandón		N		Martín *et al*. 2006
X	Mencía	Jaen	N	Tinta Pinheira (P)	Martín *et al*. 2006
X	Merenzao	Bastardo	N	María Ordoña (S), Bastardillo Chico (S), Trousseau (F)	Martín *et al*. 2006
X	Mollar Cano	Negra Mole	N	Negramoll (S), Negra Mole Tinta (P)	Martín *et al*. 2006
X	Moscatel de Grano Menudo	Moscatel de Bago Miúdo	B	Moscatel Galego (P), Muscat à Petits Grains (F), Muscat Frontignan (F)	Martín *et al*. 2003
X	Negreda		N		This study (Tab.2)
X	Palomino	Malvasia Rei	B	Jerez (S), Malvazia (P)	Martín *et al*. 2003
X	Pan y Carne		N	Estaladiña (S)	This study (Tab. 2)
X	Pedrol	Pedral	N		Martín *et al*. 2006
P		Periquita	N		This study (Tab. 2)
X	Prieto Picudo Tinto		N		Martín *et al*. 2003
X	Puesta en Cruz	Rabigato	B	Hombros (S)	Martín *et al*. 2006
X	Puesto Mayor	Saborinho	N	Verdejo Tinto (S)	Martín *et al*. 2006
X	Río Abaixo		B		This study (Tab. 2)
X	Rufete	Tinta Pinheira	N	Rufeta (S)	Martín *et al*. 2006
P		Sousao Galego	N		This study (Tab. 2)
X	Sousón	Vinhão	N	Loureira Tinta (S), Sousao (P), Albarello (P)	Martín *et al*. 2006
X	Tempranillo	Tinta Roriz	N	Aragonez (P), Arauxa (P)	This study (Tab. 2)
E	Temprano Blanco		B	Chasselas Doré (F)	Martín *et al*. 2003
P	Torrontés portugués	Terrantez	B	Terrantés (P)	Martín *et al*. 2006
X	Tinta Jeromo		N		Martín *et al*. 2006
P	Torrontés	Boal Cachudo	B	Malvasía Fina (P), Arinto (P),	Martín *et al*. 2006
E		Traminer	B	Savagnin Blanc (F)	This study (Tab. 2)
X	Treixadura	Trajadura	B	Trincadeira (P)	Martín *et al*. 2006
E	Ugni Blanc	Tália	B	Trebbiano Toscano (I), Douradinha (P)	This study (Tab. 2)
X	Verdejo		B	Verdeja (S)	Martín *et al*. 2003
X	Verdejo Colorado		RG		Martín *et al*. 2006
X	Verdejo Serrano		B		This study (Tab. 2)

[1] X = cited by García de los Salmones (1914); P = portuguese varieties; E = foreign varieties from other European countries outside Spain and Portugal. [2] B = white; N = black; RG = red. [3] F = France; S = Spain; P = Portugal; I = Italy.

8.3. The highest frequency occurs in the case of allele 237 for microsatellite VVMD7. Only two unique alleles were detected, 191 and 253 for microsatellites ssrVrZAG62 and ssrVrZAG79 respectively. In all cases prime or main names were recommended for each variety. In many instances, two names, in Spanish and in Portuguese are indicated (Tab. 1). Also the detected synonymies in the studied accessions are included (Tab. 1). Some of them can originate homonymies, *i.e.* the same name applied to different varieties, thus causing possible errors in the identification of grapevine varieties. Within the white varieties, 'Alvarinho' in Portugal, that is the spanish 'Albariño' is different from 'Alvarinhao', a synonymie of 'Caiño Blanco'. 'Verdejo' or 'Verdejo Blanco', a synonymie of 'Godello' has a homonymie with

Table 2

Allele sizes in base pairs found in some varieties identified in this study, using 6 microsatellite loci. The profiles of the other identified varieties have been previously published by our own research group (see Tab. 1)

Identified variety[1]	Berry Colour[2]	VVS2		VVMD5		VVMD7		VVMD27		ssrVrZAG62		ssrVrZAG79	
Aramon	N	130	140	230	230	237	241	177	191	187	195	241	255
Cagarrizo	B	130	150	228	236	241	245	181	185	193	203	243	249
Negreda	N	134	150	222	228	237	261	177	181	187	193	245	257
Pan y Carne	N	140	150	222	234	251	255	177	185	193	199	243	259
Periquita	N	140	142	232	234	241	255	175	177	187	187	245	249
Pinot Noir	N	**134**	**148**	**224**	**234**	**237**	**241**	**181**	**185**	**187**	**193**	**237**	**243**
Río Abaixo	B	130	142	230	236	241	255	177	185	187	193	241	245
Sousao Galego	N	132	140	222	228	237	249	175	177	185	193	245	249
Tempranillo	N	**140**	**142**	**232**	**232**	**237**	**251**	**179**	**179**	**195**	**195**	**245**	**249**
Traminer	B	150	150	228	234	241	255	185	185	187	193	243	249
Ugni Blanc (Trebbiano Toscano)	B	130	140	222	228	247	251	175	179	193	199	243	249
Verdejo Serrano	B	134	156	222	232	237	247	177	181	187	203	245	257

[1] 'Pinot Noir' and 'Tempranillo' were added as references. [2] B = white; N = black.

Table 3

Allele size (AS) in base pairs and allele frequencies (AF) found in the 53 identified varieties (see Tab. 1) for the 6 microsatellite loci. Bold numbers indicate the alleles and the value of the highest frequency for each microsatellite. Italic numbers are the unique alleles and their frequencies

VVS2		VVMD5		VVMD7		VVMD27		ssrVrZAG62		ssrVrZAG79	
AS	AF	AS	AF	AS	AF	AS	AF	AS	AF	AS	AF
130	0.198	218	0.066	231	0.028	171	0.028	185	0.170	237	0.028
132	0.047	**222**	**0.236**	**237**	**0.443**	175	0.113	**187**	**0.330**	241	0.038
134	0.047	224	0.085	241	0.094	177	0.208	*191*	*0.009*	243	0.094
136	0.019	228	0.142	245	0.047	179	0.085	193	0.217	245	0.255
140	0.217	230	0.066	247	0.085	181	0.170	195	0.132	247	0.019
142	0.132	232	0.132	249	0.028	**185**	**0.302**	199	0.047	**249**	**0.396**
150	**0.255**	234	0.189	251	0.057	191	0.094	203	0.094	*253*	*0.009*
154	0.019	236	0.085	255	0.151					255	0.075
156	0.066			261	0.066					257	0.066
										259	0.019

the spanish 'Verdejo'. Also the 'Bastardo Ruzo', synonymie of 'Godello' is not the same than the portuguese 'Bastardo' or 'Merenzao'. 'Malvasía Blanca' or 'Doña Blanca', is often named only 'Malvasía', which may leads to errors since there are several varieties with the same name. In the region of Extremadura (Spain) it is known as 'Cigüente'. In some places of the region of Castilla y León it is called 'Malvasía Castellana'.

'Palomino' or 'Jerez' is cultivated in several places of Galicia as well as in other localities in northwest Spain. In Portugal 'Palomino' is called 'Malvasia Rei', or 'Malvazia' in the region of Dão. It is the variety grown in Jerez and in some other places of Andalucía in Spain, under the same names.

'Moscatel de Grano Menudo' or 'Moscatel Galego' is a variety known since ancient times. There is another variety with red or reddish berries that is called 'Moscatel de Grano Menudo rojo', named 'Moscatel Roxo' in Portugal, with the same allele values for the studied microsatellites. From the ampelographic point of view, the only detected difference is the color of the berries. 'Torrontés' is a variety with several homonymies that refer to different varieties. The 'Torrontés' that is mainly grown in Galicia is the variety called 'Boal Cachudo' in Portugal, also named 'Malvasía Fina' in some regions of north Portugal, or Arinto in some places, although the last name has several homonymies, hence leading to misidentifications. There is also a portuguese variety denominated 'Terrantez' or 'Terrantés', that has been detected in several vineyards in Galicia, that has been called 'Torrontés Portugués'. On the other side, there is a 'Torrontés', the 'Torrontés de Montilla', a synonymy of 'Zalema', present in several places of Andalucía that is a different variety. Moreover, in La Rioja there is a 'Turruntés' or 'Torrontés', which is also a different variety (Rodríguez-Torres *et al.* 2000).

With respect to the black or red varieties, 'Brancellao' in Portugal is denominated 'Alvarelhao', that should be distinguished from 'Albarello', a synonymie of 'Sousón'.

Besides the 'Caiño Tinto', there are other 'Caiño' that are different, and with a lesser expansion (Santiago *et al.*

2005 a); among them it should be mentioned 'Caiño Bravo', from Galicia, that is the 'Amaral' or 'Azal Tinto' from Portugal (Santiago *et al.* 2005 b).

Espadeiro is known with this name in Galicia and in Portugal; in the last country it is also denominated 'Padeiro' in some places, a variety that is different from 'Padeiro da Basto'.

'Mencía', cultivated in Galicia, El Bierzo (León) and in some other regions of northwest Spain is the variety known as 'Jaen' in Portugal. It should not be mistaken with the Spanish white variety called 'Jaén' or 'Jaén Blanco'.

A recent study of twenty-two cultivars also from the north-western Iberian peninsula (Gago *et al.* 2009), carried out both by ampelography and microsatellites, including nine of the varieties in Tab. 1, mostly based on the molecular markers arrives to the preliminary identification of 'Agudelo' and 'Chenin Blanc', 'Dona Blanca' and 'Cigüente', and 'Brancellao' and 'Alvarelhao' in coincidence with the present work.

As it can be observed in the list of varieties (Tab. 1), in most cases the names in Spain and in Portugal are different. In the present study also several synonymies not previously cited have been detected. On the other side, there are synonymies that can generate homonymies, since the same name can refer to different varieties; this fact can lead to errors or misidentifications. Consequently the use of specific or prime names for each variety is recommend. Tab. 1 summarizes these proposed names, both in Spain and in Portugal, for denomination of the studied varieties.

The use of molecular markers is recommended, in particular the OIV 801 to 806 microsatellite descriptors, plus ampelographic characters, in order to identify the true-to-type grapevine varieties. In this way a precise identification can be carried out and misidentifications can be avoided when grapevine varieties are sampled in the vineyards. The minor varieties detected in the present study should be preserved in germplasm banks in order to prevent their extinction and maintain the biodiversity of the region.

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