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Ampelography and microsatellite DNA analysis of autochthonous and endangered grapevine cultivars in the province of Huesca (Spain)

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Abstract

The province of Huesca (Spain) is a traditional area for growing grapevines. There are archaeological evidences of their cultivation at least since the second century B.C. Nevertheless, in the last years the varietal diversity is markedly decreasing due to the rooting out of old plantations that are replaced with new broadly known varieties, since the market demands a certain standardization of the wine producing varieties. The objective of this study is to examine and characterise the existing varieties in the province of Huesca, mainly in vineyards planted before 1960. A total of 47 accessions were collected in 14 plots from 11 municipalities. Moreover 36 accessions from the region, planted at the Movera grapevine germplasm bank (Movera collection), located at Zaragoza (Spain) were included in the study. Ampelographic characterisation was carried out with 50 OIV descriptors for two consecutive years. Molecular characterisation was made with the following microsatellites: VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79. Ampelographic results were compared by cluster analysis. Most of the accessions were identified, being considered as new varieties: two white, Carrillera and Moscatel, and six red or black: Macicillo, Bomogastro, Angelina, Terrer, Parraleta Roja and Garnacha Gorda. Several new synonymies were detected, as Miguel for Vitadillo or Parrel for Trepat. Seven accessions remained unidentified. Out of the studied *Vitis vinifera* varieties, 23 were considered as endangered, indicating the valuable patrimony of the zone to be preserved.

Additional key words: homonymy; minor varieties; synonymies; varietal identification; *Vitis* germplasm bank.

Resumen

Ampelografía y análisis de ADN con microsatélites de cultivares de vid autóctonos y en peligro de extinción en la provincia de Huesca (España)

La provincia de Huesca (España) ha sido un lugar tradicional de cultivo de vid. Existe evidencia arqueológica de la existencia de dicho cultivo desde el siglo II a.C. Sin embargo, en las últimas décadas la diversidad varietal está disminuyendo marcadamente debido al arranque de antiguas plantaciones que están siendo reemplazadas con nuevas variedades ampliamente conocidas, ya que el mercado demanda una cierta estandarización de las variedades productoras de vino. El objetivo de este estudio es caracterizar e identificar las variedades existentes en la provincia de Huesca, principalmente en viñedos plantados antes de 1960. Se muestrearon 47 accesiones de 14 parcelas en 11 municipios. Además se incluyeron otras 36 accesiones de la provincia de Huesca que se encuentran en el banco de germoplasma de vid de Movera (colección de Movera), situado en Zaragoza (España). La caracterización ampelográfica se llevó a cabo con 50 descriptores durante dos años consecutivos. La caracterización molecular se realizó con los siguientes microsatélites: VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 y VrZAG79. Los resultados ampelográficos se compararon mediante análisis de enjambres. Se identificó la mayor parte de las accesiones, detectándose las siguientes variedades nuevas: dos blancas, Carrillera y Moscatel, y seis rojas o negras, Macicillo, Bomogastro, Angelina, Terrer, Parraleta Roja y Garnacha Gorda. Se detectaron varias sinonimias nuevas, como Miguel para Vitadillo o Parrel para Trepat. Siete accesiones quedaron sin identificar. De las variedades de *Vitis vinifera* estudiadas, 23 se consideraron en peligro de extinción, lo que indica el valioso patrimonio de la zona que debe conservarse.

Palabras clave adicionales: banco de germoplasma de vid; homonimia; identificación varietal; sinonimias; variedades minoritarias.

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Introduction

The province of Huesca (Spain) is located at the Northeast of the Iberian Peninsula, south of the central Pyrenees. Remains of grapevine plantations from the 2nd century have been found in the region, where this cultivation has been an economical basis of the agriculture since (Dupré, 1990).

In the ancient times the plantations expanded from the Pyrenees southward to the Ebro river. The surface of vineyards was subsequently reduced due to legal ordinances during the Roman domination and to religious reasons during the Muslim occupation. Along the 11th century, grapevine cultivation was again expanded throughout the whole region, linked to the monasteries that yielded land to the farmers for grapevine plantations (Lorente and Barbacil, 1994). Along this period the varietal exchange increased favoured by the St. James Way (Lavignac, 2001).

Latorre (2001) mentions that during the 17th century started the creation of quality regions like the one around the nowadays DO (denomination of origin) Somontano. In the 18th century the modern cultural practices were already applied in the province, and the obtained wine was a high quality product, expensive, and demanded in the markets (Estella, 1981).

The outbreak of phylloxera (*Viteus vitifoliae*, Fitch) in France in the 19th century caused an increase in the grapevine plantations in Huesca, reaching a total of 150,000 ha in 1885, as well as the introduction of some French varieties (Sabio, 2001). This situation however lasted only for a few years, since the pest was introduced in the region around 1900, causing the massive uproot of vineyards, being reduced the surface to about 50,000 ha by 1910. From that time on grapevine cultivation evolved into a marginal product for self consumption, with low quality and surface. In the 1980 decade, the 'DO Somontano' was created and a new stage for viticulture in the region started. At the present there are 6,000 ha included in the DO, most of them with internationally accepted varieties. The traditional varieties of the province have been mostly neglected, and the ancient grapevine patrimony almost lost. Moreover, legal specifications have promoted the uprooting of old plots and the consequent loss of ancient varieties.

The main objective of the present study is the detection, identification and recovering of endangered ancient grapevine varieties of the zone. With this aim, a broad prospection was carried out in old vineyards throughout the province of Huesca (Spain). The collected plant material plus the accessions of the zone located at the Movera grapevine germplasm bank at Zaragoza (Spain) (Movera collection), were studied in order to be identified and eventually added to the germplasm bank for conservation.

Material and methods

The prospection was carried out by checking the grapevine plantations that were established prior to 1960 in the province of Huesca (Spain). As a result, 14 plots were selected, within 11 localities, and including a total of 47 accessions. Moreover, 36 accessions from the Movera collection, previously collected in the zone, were added to the study. Table 1 summarizes the plant material, with their origin, local names and berry colour, being all of them cultivars of *Vitis vinifera*.

Ampelography

Ampelographic description was carried out for two consecutive years, 2005 and 2006, at each studied plot. Observations were made four times per season, at the following phenological stages: shooting, flowering, colour break and ripening. The observed descriptors correspond to the OIV (2009) catalogue. A total of 50 descriptors were included, that correspond to the ones proposed by Chávez (2000): 4 in young shoots, 7 in sarments, 2 in tendrils, 3 in young leaves, 17 in adult leaves, 1 in inflorescences, 5 in bunches and 11 in berries. The statistical modes were the values used for characterization of the studied accessions. A dendrogram of the studied varieties was obtained by the UPGMA (Rohlf, 1998), using the average taxonomic distances.

Microsatellites

At springtime, when the plants have shoots shorter than 15 cm, young leaves were sampled and kept at –20°C until the time of analysis. The number of samples varied from 3 to 5 for each accession.

Abbreviations used: D (discrimination power); DO (denomination of origin); He (expected heterozygosity); Ho (observed heterozigosity); Ne (number of effective alleles); PI (probability of identity); PIC (polymorphic information content).

Table 1. List of the studied accessions

Plot code[1]	Origin[2]	Local name	Berry colour[3]	Identified variety[4]
1	Alcubierrre	Blanquera	B	**ALCAÑÓN**
1	Alcubierre	Garnacha	N	GARNACHA
1	Alcubierre	Parrel	N	DERECHERO
2	Angüés	Garnacha	N	GARNACHA
2	Angüés	Macabeo	B	MACABEO
2	Angüés	Moristel	N	MORISTEL
3	Angüés	Garnacha	N	GARNACHA
3	Angüés	Moscatel	B	**MOSCATEL DE ANGÜÉS**
4	Aniés	Ribote	N	**PARRALETA**
5	Arascués	Garnacha	N	GARNACHA
5	Arascués	Parraleta roja	N	**PARRALETA ROJA**
5	Arascués	Ribote	N	**PARRALETA**
5	Arascués	Vitadillo	N	**VIDADILLO**
6	Arén	Monastrell	N	MONASTRELL
7	Ayerbe	Miguel de Arco 1	N	**MIGUEL DE ARCO**
7	Ayerbe	Miguel de Arco 2	Rg	**ROJAL**
7	Ayerbe	Miguel de Arco 3	N	**MIGUEL DE ARCO**
7	Ayerbe	Cabernet Sauvignon	N	CABERNET SAUVIGNON
8	El Grado	Alcañón	B	**ALCAÑÓN**
8	El Grado	Garnacha fina	N	GARNACHA
8	El Grado	Garnacha gorda	N	**GARNACHA GORDA (homonymy)**
8	El Grado	Moristel	N	MORISTEL
8	El Grado	Parrel	N	**TREPAT**
8	El Grado	Terrer	Rg	**TERRER**
9	Gurrea	Garnacha	N	GARNACHA
10	La Cabezonada	Bomogastro	N	**BOMOGASTRO (homonymy)**
10	La Cabezonada	Salceño	B	**SALCEÑO**
11	La Cabezonada	Alcañón	B	**ALCAÑÓN**
11	La Cabezonada	Carrillera	B	**CARRILLERA**
11	La Cabezonada	Macabeo	B	**ALCAÑÓN**
11	La Cabezonada	Miguel	N	**VIDADILLO**
11	La Cabezonada	Parrel Verdal	N	**TREPAT**
12	Loporzano	Alcañón	B	**ALCAÑÓN**
12	Loporzano	Garnacha	N	GARNACHA
12	Loporzano	Ribote	N	**PARRALETA**
13	Loporzano	Alcañón	B	**ALCAÑÓN**
13	Loporzano	Garnacha	N	GARNACHA
13	Loporzano	Greque	B	**ALCAÑÓN**
13	Loporzano	Moscatel	B	**MOSCATEL DE ANGÜÉS**
13	Loporzano	Ribote	N	**PARRALETA**
13	Loporzano	Royal	Rg	**ROJAL**
14	Secastilla	Alcañón	B	**ALCAÑÓN**
14	Secastilla	Garnacha basta	N	**VIDADILLO**
14	Secastilla	Garnacha catalana	N	MANDÓN
14	Secastilla	Garnacha	N	GARNACHA
14	Secastilla	Moscatel	B	**MOSCATEL DESCONOCIDO**
14	Secastilla	Parrel	N	**TREPAT**
M	Alcubierre	Unidentified	N	PINOT
M	Alcubierre	Unidentified	N	**PARRALETA**
M	Alcubierre	Unidentified	B	**Unidentified 1**
M	Alcubierre	Unidentified	B	**Unidentified 2**
M	Almudévar	Parraleta	N	**PARRALETA**
M	Almudévar	Unidentified	N	BENEDICTO

Table 1 (cont.). List of the studied accessions

Plot code[1]	Origin[2]	Local name	Berry colour[3]	Identified variety[4]
M	Almudévar	Unidentified	N	**PARRALETA**
M	Almudévar	Unidentified	N	**PARRALETA**
M	Almudévar	Unidentified	N	**PARRALETA**
M	Almudévar	Unidentified	N	**Unidentified 3**
M	Almudévar	Unidentified	N	MONASTRELL
M	Arascués	Garnacha	N	GARNACHA
M	Castejón	Parraleta	N	MANDÓN
M	Junzano	Macicillo	N	**MACICILLO**
M	Junzano	Parrel	N	**TREPAT**
M	Junzano	Royal	Rg	**ROJAL**
M	Longares	Garnacha	N	GARNACHA
M	Provedo	Mazuela	N	MAZUELA
M	Salas Altas	Angelina	N	**ANGELINA**
M	Salas Altas	Macabeo	B	**Unidentified 4**
M	Salas Altas	Unidentified	N	**Unidentified 5**
M	Salas Altas	Unidentified	B	**SALCEÑO**
M	Salas Altas	Unidentified	B	ALARIJE
M	Salas Altas	Moscatel	B	**MOSCATEL DE ANGÜÉS**
M	Salas Altas	Unidentified	B	MOSCATEL ALEJANDRÍA
M	Salas Altas	Unidentified	B	**Unidentified 6**
M	Salas Altas	Moscatel GM	B	MOSCATEL G. MENUDO
M	Salas Altas	Vidadillo	N	**VIDADILLO**
M	V. de Sigena	Unidentified	B	MOSCATEL ALEJANDRÍA
M	V. de Sigena	Unidentified	B	XARELLO
M	V. de Sigena	Unidentified	N	**MIGUEL DE ARCO**
M	V. de Sigena	Unidentified	Rg	**ROJAL**
M	V. de Sigena	Unidentified	B	MOSCATEL ALEJANDRÍA
M	V. de Sigena	Unidentified	B	**Unidentified 7**
M	V. de Sigena	Unidentified	B	MOSCATEL ALEJANDRÍA
M	Valle de Hecho	Unidentified	B	CASTELLANA BLANCA

[1] M: accessions previously included in the Movera (Zaragoza) germplasm bank. [2] Name of the locality in the province of Huesca (Spain), except for two accessions: *Longares* (Zaragoza) and *Provedo*, from Viveros Provedo at La Rioja. [3] B: white; Rg: red; N: black; according to OIV (2009) catalogue. [4] In bold letters, varieties in danger of extinction (see Table 2) .

DNA was extracted from the frozen leaves by using MasterPure™ Plant Leaf DNA Purification Kit (Epicentre Technologies, Madison, Wis.). Extracted DNA was quantified and a working solution of DNA (10 ng μL^{-1}) was made.

A total of six microsatellite loci fully characterized in previous studies were used: VVS2 (Thomas and Scott, 1993); VVMD5 and VVMD7 (Bowers *et al.*, 1996); and VVMD27 (Bowers *et al.,* 1999), equivalent to VrZAG47 with a difference of 10 base pairs; and VrZAG62 and VrZAG79 (Sefc *et al.*, 1999). These microsatellites were selected based on previous studies (Martín *et al.*, 2003), and they correspond to OIV801 trough OIV806 descriptors (OIV, 2009).

Amplification reactions were carried out in a thermocycler iCycler (Bio-Rad), with a final volume of 20 µL, and a concentration of 1X EcoTaq Buffer (Ecogen), 2 mM $MgCl_2$, 200 µM for each dNTP (Ecogen), 1 U Ecotaq DNA Polymerase (Ecogen) and the following concentrations for each primer: 0.1 µM for VrZAG62, 0.2 µM for VVS2, 0.25 µM for VVMD7 and 0.5 µM for VVMD5, VVMD27 and VrZAG79 (Bonsai Technologies Group S.A.). Temperature program used was as follows: 5 min at 95°C, 40 cycles of 45 sec at 94°C, 1 min at 50°C, 1 min 30 sec at 72°C and a final extension of 7 min at 72°C.

The amplified fragments, marked with fluorescence, were electrophoretically separated and detected in an automatic sequencer for DNA MegaBACE500 (GE HealthCare). The allele size, expressed in base pairs, was determined with the software Fragment Profiler v.1.2 (GE HealthCare).

The obtained genotypes were compared to existing publications and databases for identification of the accessions (Martín *et al.*, 2003; VIVC, 2007; SIVVEM, 2008; Gago *et al.,* 2009; Castro *et al*., 2010; Zaharieva *et al.,* 2010).

The number of effective alleles (Ne) was calculated according to Brown and Weir (1983). The observed heterozigosity (Ho) is the ratio between the heterozygous genotypes and the total of genotypes analyzed for each locus. The expected heterozygosity (He) was obtained by the formula of Nei (1987). Discrimination power (D) was calculated according to Tessier *et al.* (1999). The polymorphic information content (PIC), according to Kalinowski *et al.* (2007). The frequency of null alleles was calculated following Wagner and Sefc (1999). The used software for calculations was GENALEX (Peakall and Smouse, 2006). The probability of identity (PI) is the probability for two random samples to have the same genotype (Paetkau *et al.*, 1995). It was obtained with the software IDENTITY 1.0 (Wagner and Sefc, 1999).

Results

As a result of the ampelographic plus the microsatellite characterization, most of the studied accessions were identified with known grapevine varieties (see Table 1), by using the references indicated in the former section. The varietal names were assigned based on the comparison with available microsatellite databases, plus by observation of the ampelographic descriptors. Thirty one accessions corresponded to 15 major varieties, while 45 were minor previously reported or newly identified varieties (in bold letteres en Table 1). Seven accessions remained as unidentified.

The last two groups of accessions, minor or newly identified plus unidentified, are the ones considered in danger of extinction, with little or none references. Table 2 lists these varieties, indicating the number of sampled accessions for each. Eight of them were detected in more than one location, and they correspond to old autochthonous varieties. These are: Alcañón, Miguel de Arco, Moscatel de Angüés, Parraleta, Rojal, Salceño, Trepat and Vidadillo. Another eight accessions were detected in only one plot: Bomogastro and Garnacha Gorda, that are two cases of homonymies, Angelina, Carrillera, Macicillo, Parraleta Roja, Terrer and one Moscatel that does not correspond to none known variety of Moscatel. These 16 varieties plus the 7 unidentified ones were the object of the ampelographic and microsatellite characterization. All of them were considered with a more or less marked risk of extinction.

Table 2. List of endangered varieties collected at the province of Huesca (Spain)

Variety	Number of accessions	Local names
ALCAÑÓN	8	Alcañón, Blanquera, Greque
ANGELINA	1	Angelina
BOMOGASTRO (homonymy)	1	Bomogastro
CARRILLERA	1	Carrillera
GARNACHA GORDA (homonymy)	1	Garnacha gorda
MACICILLO	1	Macicillo
MIGUEL DE ARCO	3	Miguel de Arco
MOSCATEL DE ANGÜÉS	3	Moscatel
MOSCATEL	1	Moscatel
PARRALETA	9	Parraleta, Ribote
PARRALETA ROJA	1	Parraleta roja
ROJAL	4	Royal
SALCEÑO	2	Salceño
TERRER	1	Terrer
TREPAT	4	Parrel, Parrel Verdal
VIDADILLO	4	Vidadillo, Vitadillo, Garnacha basta, Miguel
Unidentified 1	1	None
Unidentified 2	1	None
Unidentified 3	1	None
Unidentified 4	1	None
Unidentified 5	1	None
Unidentified 6	1	None
Unidentified 7	1	None

Table 3. OIV descriptors in sarment, tendrils and young leaf for collected endangered varieties (data from two years)

Identified variety	Young sarment				Sarment							Tendrils		Young leaf		
	OIV1	OIV2	OIV3	OIV4	OIV6	OIV7	OIV8	OIV9	OIV10	OIV11	OIV15	OIV16	OIV17	OIV51	OIV53	OIV55
ALCAÑON	7	2	5	5	1	1	1	2	2	1	1	1	5	1	5	3
ANGELINA	7	3	3	1	7	2	2	2	2	1	5	1	5	4	1	1
BOMOGASTRO (homonymy)	7	2	5	5	1	2	2	2	2	5	1	1	1	3	5	7
CARRILLERA	7	2	3	1	3	1	1	1	1	1	1	1	5	4	1	1
GARNACHA GORDA (homonymy)	7	2	5	5	5	1	1	1	1	1	1	1	3	3	5	5
MACICILLO	7	2	1	1	1	2	2	2	2	1	1	1	3	3	1	1
MIGUEL DE ARCO	7	2	3	5	5	2	2	2	1	1	1	1	5	3	7	7
MOSCATEL DE ANGÜÉS	7	2	3	5	5	1	1	1	1	1	1	1	3	3	5	7
MOSCATEL	7	2	3	3	5	1	1	1	1	1	1	1	3	4	3	1
PARRALETA	7	3	7	3	1	2	2	2	2	1	5	1	5	1	7	3
PARRALETA ROJA	7	3	7	5	1	2	2	2	2	1	5	1	3	4	5	7
ROJAL	7	2	1	1	3	1	1	1	1	1	1	1	3	3	1	1
SALCEÑO	7	2	7	5	3	1	1	2	2	5	5	1	3	3	5	7
TERRER	7	2	3	3	3	1	1	2	2	1	1	1	2	4	5	5
TREPAT	7	2	5	3	1	1	1	1	1	5	1	1	7	1	7	7
VIDADILLO	7	2	5	3	3	2	2	2	2	1	5	1	2	3	1	1
Unidentified 1	7	2	1	1	5	2	2	2	2	1	1	1	4	4	1	1
Unidentified 2	7	2	5	5	1	2	2	1	2	3	5	1	1	1	5	5
Unidentified 3	7	2	3	3	3	1	1	2	1	1	1	1	3	3	3	1
Unidentified 4	7	2	5	5	5	1	1	1	1	7	1	1	3	1	7	9
Unidentified 5	7	2	5	3	3	1	1	2	2	3	1	1	3	3	5	5
Unidentified 6	7	2	5	3	3	2	2	2	2	1	5	1	3	3	1	1
Unidentified 7	7	2	3	5	3	2	1	1	1	1	1	1	5	3	5	7

Ampelographic results for these varieties are shown in Tables 3, 4 and 5. Descriptors OIV1 (aperture of tip), OIV16 (number of consecutive tendrils), OIV151 (sexual organs), OIV230 (intensity of the anthocyanin coloration of flesh) and OIV241 (formation of seeds), have the same values in all cases. From the agronomical point of view, one of the most important descriptors is the colour of the skin (OIV225).

All these varieties can be identified by their ampelographic descriptors. Dendrogram of the observed variables is shown in Figure 1. Some of the studied varieties have a certain closeness, like Carrillera and Rojal; Macicillo and Unidentified 3; Garnacha Gorda and Miguel de Arco, and at a lower level Parraleta and Parraleta Roja, and Moscatel de Angüés and Unidentified 7. The rest of the varieties have differences in several descriptors.

Table 6 shows the results of the DNA analysis. The combination of profiles for the six studied microsatellite loci permits to distinguish the studied varieties. A total of 23 different patterns were obtained. All the varieties had unique genotypes at least for one of the microsatellites except Alcañón, Rojal and Trepat. In the case of Macicillo, all the genotypes were unique except for MD7.

With respect to the alleles (Table 6) there were three unique alleles in Bomogastro; two in Parraleta Roja and Unidentified 6; and one in Macicillo, Miguel de Arco, Moscatel de Angüés, Salceño and Terrer.

Results of the analysed microsatellites are summarized in Table 7. The average number of alleles (Na) was 9 while the Ne had an average of 5.2. The highest PIC was 0.84 for VVMD5, and the lowest, 0.68 for VrZAG62. The Ho (Table 7) ranges from 0.78 for VrZAG62 and VVMD7 to 0.96 for VVMD27, being these values always higher than the He, that ranged from 0.72 to 0.85.

Microsatellites were able to detect synonymies that were also confirmed by ampelography. Within the endangered varieties the following local names resulted to be the same variety (Table 2): i) Vidadillo, Vitadillo, Garnacha Basta or Miguel; ii) Parraleta or Ribote, while Bomogastro, a synonymy of Parraleta, mentioned in the literature, appeared as a homonymy; iii) Parrel

Table 4. OIV descriptors in adult leaf for collected endangered varieties (data from two years)

Identified variety	Adult leaf																
	OIV65	OIV67	OIV68	OIV70	OIV72	OIV74	OIV75	OIV76	OIV79	OIV80	OIV81.1	OIV81.2	OIV82	OIV83.1	OIV83.2	OIV84	OIV87
ALCAÑON	5	3	3	1	2	2	3	5	5	3	2	1	2	3	1	5	7
ANGELINA	4	4	3	1	1	5	3	3	2	2	1	1	2	3	1	1	1
BOMOGASTRO (homonymy)	3	3	3	1	1	5	3	3	5	1	1	1	2	3	2	7	7
CARRILLERA	2	3	3	1	1	3	5	5	2	2	1	1	2	2	1	1	1
GARNACHA GORDA (homonymy)	2	4	2	1	1	5	3	5	3	1	1	1	2	2	1	5	5
MACICILLO	4	4	2	1	1	4	3	3	2	2	1	1	3	1	1	1	1
MIGUEL DE ARCO	3	3	3	1	1	5	5	5	3	2	2	1	2	3	1	5	5
MOSCATEL DE ANGÜÉS	2	3	2	1	1	5	3	3	3	3	1	1	2	3	1	5	5
MOSCATEL	2	3	3	1	1	3	3	3	4	3	1	1	2	3	1	3	1
PARRALETA	3	3	3	3	1	5	5	3	5	3	2	1	3	1	2	7	7
PARRALETA ROJA	3	3	3	3	2	5	7	5	5	3	2	1	3	1	2	7	7
ROJAL	3	4	3	1	1	5	5	3	4	3	1	1	3	3	1	1	1
SALCEÑO	2	3	3	1	1	5	3	3	4	2	1	1	2	1	2	7	7
TERRER	2	3	3	1	1	3	3	5	3	2	1	1	2	2	1	3	7
TREPAT	3	3	3	1	1	4	5	3	2	2	2	1	3	1	2	7	7
VIDADILLO	2	4	3	1	1	5	3	5	2	2	1	1	2	3	1	1	1
Unidentified 1	4	2	3	4	1	3	3	3	2	2	1	1	3	3	1	1	1
Unidentified 2	2	4	3	1	1	5	3	2	3	1	1	1	3	1	2	3	3
Unidentified 3	3	4	3	1	1	4	3	5	4	3	1	1	2	2	1	1	1
Unidentified 4	3	3	3	2	1	5	3	5	3	3	1	1	3	3	1	5	5
Unidentified 5	4	3	3	1	1	5	5	3	4	3	2	1	3	3	1	5	7
Unidentified 6	3	3	3	1	1	5	3	3	5	3	1	2	3	1	1	1	5
Unidentified 7	3	3	3	1	1	1	3	5	1	1	2	1	3	1	1	5	5

or Parrel Verdal, synonymies of Trepat; and iv) Alcañón, Blanquera or Greque.

Alcañón and Parraleta were detected at least in more than four different localities, being rather frequent varieties in old plantations. Bomogastro, already mentioned, and Garnacha Gorda, that are synonymies of Parraleta and Vitadillo respectively, were found under these local names, but corresponding to two cases of homonymy.

Identification with microsatellites has a good reliability, since the cumulative PI is very low (Table 7).

Discussion

The list of endangered varieties includes a total of 16 identified varieties. Seven of them, Alcañón, Miguel de Arco, Parraleta, Rojal, Salceño, Trepat and Vidadillo as well as a Moscatel from Barbastro (Huesca) that could be the Moscatel of Angüés (Table 2) were already mentioned by García de los Salmones (1914) as being present in the zone, which indicates that they probably are old autochthonous varieties. The number of accessions varies from 2 to 9. Another eight varieties with local names, had only one accession each, as well as the seven unidentified ones. Miguel de Arco (Table 6) shows a microsatellite profile different with that recently published (Zaharieva *et al*., 2010), being a new case of homonymie, to be more deeply studied. A thorough revision has been carried out in order to identify these last seven accessions, without positive results; not either references or previous information about the eight identified varieties with only one accession (Table 2) were found. These facts indicate that they are varieties in a marked risk of extinction.

Ampelographic characterization confirms the existence of the two above mentioned homonymies: Bomogastro, different from Parraleta, synonymy of Bomogastro (Martín *et al.*, 2003) in several descriptors; and Garnacha Gorda, different from Vidadillo, synonymy of Garnacha Gorda, also in several descriptors (see Tables 3, 4 and 5). These two homonymies were not previously cited.

Results of the molecular analysis evidenced the genetic differences between the pairs of varieties that are

Table 5. OIV descriptors in reproductive organs for collected endangered varieties (data from two years)

Identity variety	Inflor	Bunch					Berry										
	OVI151	OIV202	OIV203	OIV204	OIV208	OIV209	OIV220	OIV221	OIV223	OIV225	OIV228	OIV232	OIV235	OIV236	OIV230	OIV240	OIV241
ALCAÑON	3	7	5	3	1	3	3	3	4	1	5	2	2	1	1	1	3
ANGELINA	3	7	7	3	3	3	7	7	4	5	5	2	2	1	1	1	3
BOMOGASTRO (homonymy)	3	5	5	5	2	1	3	3	4	5	5	2	2	1	1	1	3
CARRILLERA	3	5	3	3	2	2	5	5	3	1	5	3	2	1	1	1	3
GARNACHA GORDA (homonymy)	3	5	5	5	2	2	5	5	4	5	5	2	2	1	1	2	3
MACICILLO	3	5	3	7	1	2	5	5	4	5	5	3	3	1	1	1	3
MIGUEL DE ARCO	3	5	7	7	2	3	3	3	4	6	5	2	2	1	1	1	3
MOSCATEL DE ANGÜÉS	3	7	5	3	2	2	5	3	4	1	5	3	2	2	1	1	3
MOSCATEL	3	7	5	5	1	2	7	7	4	1	5	2	2	1	1	1	3
PARRALETA	3	5	3	5	2	3	5	5	3	6	5	3	2	1	1	1	3
PARRALETA ROJA	3	5	5	7	2	3	5	5	3	6	3	3	2	1	1	1	3
ROJAL	3	7	3	3	1	2	5	5	4	3	5	3	2	4	1	1	3
SALCEÑO	3	3	3	3	1	2	5	5	4	1	5	3	2	1	1	1	3
TERRER	3	7	7	7	2	3	5	5	3	3	3	3	2	1	1	1	3
TREPAT	3	5	5	5	2	2	5	5	3	6	5	2	2	1	1	1	3
VIDADILLO	3	5	3	3	2	2	5	5	4	5	5	3	2	1	1	1	3
Unidentified 1	3	9	3	3	1	2	7	2	2	1	5	3	2	1	1	1	3
Unidentified 2	3	3	3	3	1	1	5	5	4	1	3	2	2	1	1	1	3
Unidentified 3	3	5	5	5	2	1	5	5	3	5	5	2	2	1	1	2	3
Unidentified 4	3	7	3	3	2	3	5	5	4	1	5	3	2	1	1	1	3
Unidentified 5	3	7	5	3	2	2	7	7	4	5	5	3	1	1	1	1	3
Unidentified 6	3	5	5	3	2	2	5	5	2	1	3	2	2	1	1	1	3
Unidentified 7	3	5	5	1	2	2	5	5	3	1	5	3	2	2	1	2	3

closer in the dendrogram of Figure 1. Carrillera and Rojal differ in 8 alleles; Macicillo and Unidentified 3, also in 8 alleles; and Garnacha Gorda and Miguel de Arco in 7 alleles. Parraleta and Parraleta Roja, although having coincidence in a certain number of descriptors, present differences in characters of several organs.

There are two varieties, Alcañón and Unidentified 5 that have coincidence in all loci except two of them, which indicates a probably close relationship among them. Also the following nine pairs of varieties share at least one allele at each locus: Alcañón-Angelina; Alcañón-Rojal; Angelina-Rojal; Moscatel de Angüés-Rojal; Miguel de Arco-Trepat; Trepat-Unidentified 7; Alcañón-Unidentified 5; Angelina-Unidentified 5; and Rojal-Unidentified 5. This fact is an indication of the possibility of having a common parental for each of these pairs. Four varieties are probably more implied in potential crosses; these are Rojal, Angelina, Alcañón, and the Unidentified 5. A larger number of microsatellites would be needed in order to find out the possible crosses that originated the studied varieties.

Martín *et al.* (2003) obtained rather similar values for the Ho of the studied microsatellites, except for VVMD27 (VrZAG47), with 0.86 instead of 0.96, and VrZAG79, with 0.80 instead of 0.87 (Table 7). Moussaoui (2005) obtained values of 0.61 for VVMD7 and 0.86 for VrZAG79. In Santana *et al.* (2010), the values ranged from 0.82 to 0.91, being very close in the case of VrZAG79, 0.872 *vs* 0.870 and somewhat different for VVS2, 0.914 *vs* of 0.826, but the average values were close each other, 0.866 *vs* 0.848.

The values of the Ho were always higher than those of the He. This fact can be explained, according to Lopes *et al.* (1999), by the artificial selection in order to improve the yields and quality; or perhaps, as suggested in Santana *et al.* (2008), by the intention to maintain variability for satisfying preferences of the consumers in different regions.

With respect to the He, the obtained values, with a mean of 0.80, were almost coincident with those obtained by Martín *et al.* (2003), 0.806, Moussaoui (2005), 0.78, and similar to Santana *et al.* (2010), 0.828.

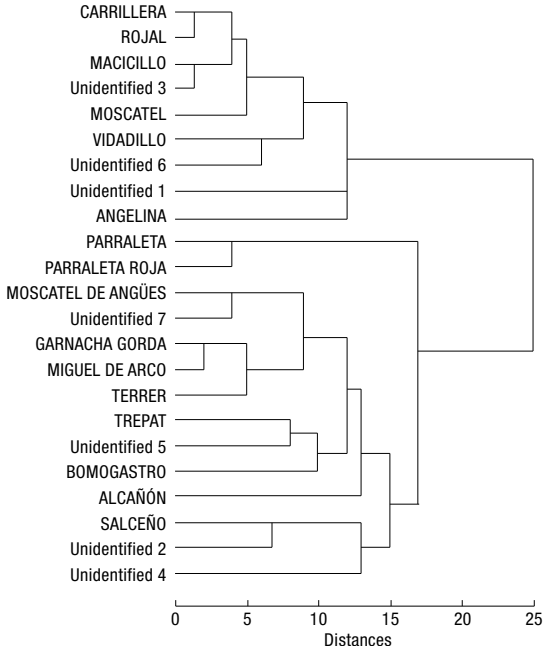

Figure 1. Dendrogram of the morphological descriptors for the endangered varieties, obtained by the UPGMA, using the average taxonomic distances.

The Ne varied from 6.83 for VrZAG79 to 3.54 for VrZAG62. Lopes *et al.* (1999) and Sefc *et al.* (2000) obtained the highest value, 7.7 for loci VVMD5, VVS2 and VrZAG79 in grapevine varieties from different zones in Europe.

As conclusions, prospection and characterization of the studied plant material has permitted the localization in the field of grapevine varieties that were important at the end of the 19th century, and that were considered as extinct at the present, as Miguel de Arco or Moscatel de Angüés. Also some new synonymies like Miguel for Vitadillo or Parrel for Trepat were found. The endangered cultivars detected in this study have been made available for inclusion in the Movera germplasm bank.

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Table 6. Allele sizes of the analysed microsatellites in the endangered varieties[1]. Unique genotypes are underlined. Unique alleles are in bold letters.

Identified variety	VVS2		VVMD5		VVMD7		VVMD27		VrZAG62		VrZAG79	
ALCAÑON	134	146	234	238	239	239	179	193	187	189	249	255
ANGELINA	138	146	228	234	233	239	193	193	189	205	253	255
BOMOGASTRO (homonymy)	134	**152**	228	232	249	251	177	183	**203**	205	**237**	249
CARRILLERA	134	134	228	236	239	247	181	183	187	205	241	245
GARNACHA GORDA (homonymy)	134	144	226	234	243	247	177	179	189	205	245	247
MACICILLO	144	146	236	236	239	239	181	187	**183**	189	243	249
MIGUEL DE ARCO	144	154	222	234	**237**	249	177	193	189	205	251	255
MOSCATEL DE ANGÜÉS	146	**150**	232	234	243	251	177	193	189	205	255	255
MOSCATEL	134	146	228	236	239	249	177	189	187	189	253	257
PARRALETA	134	134	222	238	239	239	177	187	187	189	249	259
PARRALETA ROJA	134	144	228	234	243	247	177	179	**177**	**179**	247	247
ROJAL	138	146	228	234	239	243	183	193	189	189	245	255
SALCEÑO	**148**	154	236	240	239	247	177	187	187	205	249	257
TERRER	134	134	226	226	**241**	245	177	183	189	205	247	249
TREPAT	134	144	226	234	243	249	177	193	189	205	249	255
VIDADILLO	138	154	238	240	233	239	179	193	205	205	245	255
Unidentified 1	138	144	226	238	249	251	181	193	187	205	247	257
Unidentified 2	138	146	236	240	239	249	181	193	187	205	257	259
Unidentified 3	134	158	226	226	239	239	177	181	187	187	249	251
Unidentified 4	134	146	234	236	239	239	185	187	189	189	241	241
Unidentified 5	134	146	234	238	239	245	179	193	189	189	249	255
Unidentified 6	134	134	**224**	238	243	247	187	193	**181**	189	249	255
Unidentified 7	144	158	234	236	239	243	183	193	187	189	245	249
Reference variety												
CABERNET SAUVIGNON	136	148	232	240	239	239	173	187	189	195	245	245

[1] Allele sizes of the rest of varieties in Table 1, can be consulted at http://www.sivvem.monbyte.com/sivvem.asp

Table 7. Results for the studied microsatellites in the 23 varieties of Table 1: number of alleles (Na); number of heterozygotic profiles (NHp); number of effective alleles (Ne); observed heterozygosity (Ho); expected heterozygosity (He); polymorphism information content (PIC); discrimination power (D); probability of identity (PI); estimated frequency of null alleles (ENA)

	Alelle size range	Na	NHp	Ne	Ho	He	PIC	D	PI	ENA
VVS2	134-158	9	19	4.600	0.826	0.783	0.761	0.888	0.0737	–0.049
VVMD5	222-240	9	20	6.531	0.870	0.847	0.836	0.922	0.0416	–0.071
VVMD7	233-251	9	18	4.318	0.783	0.768	0.752	0.888	0.0772	–0.079
VVMD27	177-193	8	22	5.398	0.957	0.815	0.786	0.945	0.0584	–0.068
VrZAG62	177-205	8	18	3.538	0.783	0.717	0.682	0.843	0.1277	–0.106
VrZAG79	237-259	11	20	6.826	0.870	0.853	0.831	0.930	0.0371	–0.124
Averages per locus		9		5.202	0.848	0.797	0.776	0.903		
Cumulative									6.5×10^{-8}	

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