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Short communication. Characterization of the relic Almuñécar grapevine cultivar

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Abstract

A prospection of Andalusian Mediterranean vineyards was carried out in search of the traditional Almuñécar grapevine, used to produce raisins of quality. An ampelographical description and a genetic characterization, using 20 nuclear microsatellite *loci,* showed that this cultivar constitutes a clone of Muscat of Alexandria. In reference to biotic stress, this cultivar has a high sensibility to powdery and downy mildews and medium sensibility to the grapevine pathogen *Botrytis cinerea*. At the present time, it constitutes a relic cultivar due to different sanitary and economical causes analyzed in the text.

Additional key words: ampelography; Muscat of Alexandria; nuclear microsatellite; raisin.

Resumen

Comunicación corta. Caracterización del relicto cultivar de vid Almuñécar

Se ha realizado una prospección del viñedo de la vertiente mediterránea de Andalucía para buscar la variedad tradicional Almuñécar, usada para la producción de pasas de calidad. La descripción ampelográfica y la caracterización genética, empleando 20 *loci* de microsatélites nucleares, han indicado que este vidueño constituye un clon de la variedad Moscatel de Alejandría. Respecto al estrés biótico, esta variedad se muestra muy sensible al oídio y mildiu, así como a *Botrytis cinerea.* En la actualidad, la variedad puede considerarse como relicta, debido a causas económicas y fitosanitarias referidas en el texto.

Palabras clave adicionales: ampelografía; Moscatel de Alejandría; microsatélites nucleares; pasa.

During the Arab domination of the Iberian Peninsula (from 8th to 15th century AC), vineyards were established in the southern Mediterranean coast to produce table grapes, raisins and alcohol (Gámez, 2004). Majority of this area was placed along the actual provinces of Málaga and Granada (Andalusia, Spain). From that age, the most usual cultivar was Muscat of Alexandria, named earlier Apiana by Plinius (1st century AC). Raisin production (*zebid almonacabi*, in old Spanish Arab documentation) was an important economic support for Almuñécar (Granada) and Málaga (Mármol, 1991; Marín *et al*., 1992). When this village was conquered by Castillian troops in 1489, there were 17 aranzadas (about 76 ha) of vineyard (Calero, 1984). In the age of the Enlightenment, De Torres (1785) published a book where several practices to optimize the management of the Muscat vineyards in Almuñécar municipality, focused on raisin production, are indicated. García de la Leña (1792) has a reference on the use of that cultivar in Málaga province to produce also a wine mixtured with Pero Ximen must. Clemente y Rubio (1807) indicated the great biodiversity of the Granada coast vineyards, where the main cultivars were "Jaen negro de Granada, Albillo de Granada, Pedro Ximenez Zumbon, Tinto o Tintillo de Luxar, Romé negro, Montúo castellano, Pecho de perdiz, Zurumí,

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Doradillo or Jaen, Montúo perruno, Pedro Ximenez, Calona negra, Corazón de Cabrito, Casco de tinaja, Ataubí, Jaldona, Teta de negra, Moscatel menudo blanco, Moscatel menudo morado, Moscatel gordo blanco, Vigiriega de Motril, Jamí, Mollar de Granada, Ciutí or Lanxaron". He said about the named Pasa Larga in Almuñécar or Uva de Pasa in Motril, known as Almuñécar in other Andalusian areas: "This kind of raisin is payed double that the others, it is the most famous in Málaga it is dried under a sunny exposure. A great part of Almuñécar economy is based on this product". In 1930, majority of vineyards to produce raisins were situated between Almuñécar and Salobreña towns, from Taramay state to Cambrón gully. Grapes were dried in the open air in special pools made of masonry or on roofs covered, in both cases, by the plant *Genista umbellata* (L' Her) (Vigo *et al.*, 2009).

The existence of synomyns (different names for the same variety) and homonyms (different varieties with the same name) is one of the major problems in the management of germplasm collections. The identification of grape cultivars has traditionally been based on ampelography, which is the analysis and comparison of morphological characters of leaves, shoot tips, fruit clusters and berries (IPGRI-UPOV-OIV, 1997; Galet, 2000). Some genetically related cultivars are morphologically very similar and difficult to differentiate by visual comparison (Aradhya *et al*., 2003). On the other hand, intravarietal clones can differ considerably in phenotype even though they have virtually identical DNA profiles (Vignani *et al*., 1996; Riaz *et al*., 2002). Microsatellite sites are excellent markers for grapevine characterization (Sefc *et al*., 2001). In general, all plants belonging to the same cultivar (*i.e*., from a monoclonal origin) show identical genotypes in all microsatellite loci (Ibáñez *et al*., 2009).

The aim of the present paper was to characterize and recuperate the historical relic cultivar Almuñécar for its *ex situ* conservation in the germplasm bank of the IFAPA Centro Rancho de la Merced (Jerez de la Frontera, Cádiz, Spain).

A prospection to find Almuñécar grapevine was carried out from 1996 to 2002 in vineyards located from Estepona (Málaga, Spain) to Motril (Granada, Spain). Five vines with typical ampelographical characteristics of the Almuñécar cultivar, according to the ampelographical description given by Clemente y Rubio (1807), were found around the town of Almuñécar (Granada, Spain). The accessions collected were kept in the collection of the IFAPA Centro Rancho de la Merced germplasm bank (Jerez de la Frontera, Cádiz, Spain).

The plant material for the characterization was obtained from the IFAPA Centro Rancho de la Merced germplasm bank and the plot located at Almuñécar.

Ampelographic data were collected between 2003 and 2007 in the plot located at Almuñécar and 2008 and 2009 in the IFAPA Rancho de la Merced germplasm bank. At the first time, IPGRI, UPOV, OIV (1997) code was used and later descriptors were adapted to OIV code (2009). One hundred and eight descriptors were used, including descriptors relative to young shoots, young leaves, mature leaves, woody shoots, flower, inflorescence, bunches, berries and seeds. Ten readings per each descriptor were taken on five plants. Berry measurements were made at harvest using 50 berries, from 10 bunches.

Data on the phenological stages (Baillod & Baggiolini, 1993) were collected during seven years (2003-2009) in Almuñécar. The index maximum width/maximum length of 50 berries of Almuñécar cultivar was compared with 50 berries of Muscat of Alexandria using one way ANOVA (Massart *et al*., 1997).

The sensibility to diseases (*Plasmopara viticola, Erysiphe necator* and *Botrytis cinerea*) was observed in the field each year between 2003 and 2009 on the five vines located in Almuñécar using OIV descriptor (OIV, 2009). To evaluate the sensibility to both mildews under standard laboratory conditions, tests using foliar discs, with 20 repetitions of each sample, were also carried out. In the case of the powdery mildew, the method used was the indicated by OIV (2009) and for downy mildew the procedure of Staudt & Kassemeyer (1995) and Rumbolz *et al*. (2002). Foliar discs of Palomino fino cultivar and the 41B rootstock were used as reference in the test.

DNA was extracted from young leaves using DNeasy Plant Mini Kit (Qiagen). A genotypic characterization was performed for 20 nuclear microsatellite *loci* located in the 19 linkage groups of grapevine genome: VMC1b11, VMC4F3-1 (Vitis Microsatellite Consortium); VVMD5, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32 (Bowers *et al*., 1996, 1999); VVS2 (Thomas & Scott, 1993); VVIB01, VVIH54, VVIN16, VVIN73, VVIP31, VVIP60, VVIQ52, VVIV37, VVIV67 (Merdinoglu *et al*., 2005). Two multiplex PCR tests were set up to amplify the 20 microsatellite *loci* (Vargas *et al*., 2007) and amplified with an Applied Biosystems 9700 thermocycler.

Amplified products were separated in capillary electrophoresis using an automated sequencer (ABI Prism 3130, Applied Biosystems). Fluorescently labelled fragments were detected and sized using GeneMapper v. 3.7 software (Applied Biosystems) and fragment lengths were determined with the help of internal size standards (GeneScan-500 LIZTM, Applied Biosystems).

Identification of redundant genotypes was determined by comparing microsatellite genotypes with data contained in the microsatellite grapevine databases IFAPA Centro Rancho de la Merced (in preparation) and the *Vitis* Germplasm Bank at the Finca El Encín (IMIDRA, Alcalá de Henares, Spain) (Ibañez *et al*., 2009; Vargas *et al*., 2009). Genotype comparisons were carried out using Microsatellite toolkit v. 9.0 software package (Park, 2001).

The first result to remark is that Almuñécar was a widely cultivated cultivar before phylloxera infestation and at the present time is a very minoritary cultivar along the Mediterranean coast of Andalusia.

In the Axarquía area situated in the Málaga province the main synonymies were: Moscatel Real and Larga. In Granada coast (Spain): Almuñécar, Moscatelón, Moscatelona, Pasa larga, Uva de pasa and Uva de yema. Ampelographical data for the cultivar Almuñécar are shown in Table 1.

In Muscat of Alexandria the averages of the maximum width and length of the berries were 20.64 ± 0.33 mm and 26.86 ± 0.42 mm, respectively. In the case Almuñécar cultivar, 16.48 ± 0.16 mm and 29.1 ± 0.28. The width/length indexes were 0.77± 0.01 and 0.57 ± 0.01, respectively. The comparison of the analysis of de variance using Anova test indicated there were statistically significative differences between both cultivars ($F = 294.57$; $\alpha = 0.05$).

The calendar in shoot developmental stages was: Bud burst, March 15-22; flowering time, May 15-24; veraison, July 15-26; maturation, September 5-15; leaf fall, November 19-27.

Field and laboratory observations indicated that Almuñécar cultivar had a high susceptibility to powdery and downy mildews (Table 1). On the other hand, symptoms caused by *Botrytis cinerea* are very frequent on bunches mainly in October, when the first important dews took place in Almuñécar area.

Table 2 shows the allele profiles obtained for Almuñécar cultivar. The five accessions analyzed presented the same genotype at 20 microsatellite *loci* analyzed. The genotype obtained for Almuñécar cultivar was compared with the genotypes database of the germplasm bank at IFAPA Centro Rancho de la Merced (Jiménez-Cantizano, unpublished data). Cultivars Almuñécar and Muscat of Alexandria showed the same genotype combination for the 20 microsatellite *loci*: however, these cultivars had significant morphologic differences in the berries (Figure 1). This could be explained by the occurrence of somatic variants of the same cultivars in several grapevine growing areas (Bowers *et al*., 1996). Varieties are described that differ only in minor characters, such as berry pigmentation or the presence of capillaries in the leaves caused by mutations (sports), as in Garnacha (Cabezas *et al*., 2003), Cariñena, Xarel-lo (Ibáñez *et al*., 2003), or Moscatello (De Mattia *et al*., 2007). Microsatellite analysis cannot resolve these minimal differences that occur between clonal types or sports.

Muscat of Alexandria genotype was compared with the genotype obtained for Ibáñez *et al*. (2009) and Vargas *et al*. (2009), at the same 20 microsatellite *loci*, from plant accessions kept at "El Encín" germplasm collection. We obtained very similar result of microsatellite profile of Muscat of Alexandria. In 12 of the 20 microsatellite *loci* the data for identical alleles differed by 1 bp. The differences showed of the allele sizes obtained by the two different laboratories are the result of the rounding methods (This *et al*., 2004).

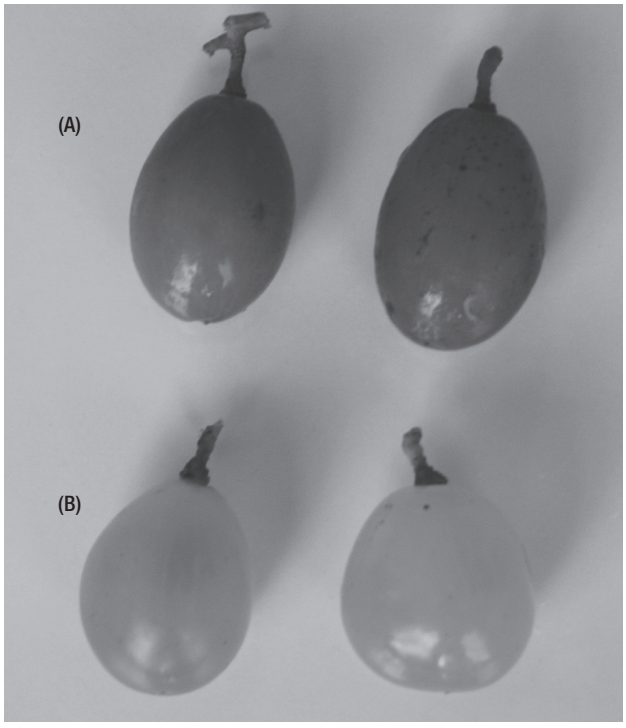

Figure 1. Morphologic differences in berries of cultivar Almuñécar (A) and Muscat of Alexandria (B).

Table 1. Mean values for the OIV (2009) ampelografic descriptors observed in Almuñécar cultivar during seven years (2003-2009)

OIV Code	Descriptor	Expression	Notes
001	Young shoot: aperture of tip	5	fully open
002	Young shoot: distribution of anthocyanin coloration on prostrate hairs of tip	2	piping
003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of tip	5	medium
004	Young shoot: density of prostrate hairs on tip	5	medium
005	Young shoot: density of erect hairs on tip	1	none or very low
006	Shoot: attitude (before tying)	1	erect
007	Shoot: colour of dorsal side of internodes	2	green and red
008	Shoot: colour of ventral side of internodes	2	green and red
009	Shoot: colour of dorsal side of nodes	3	red
010	Shoot: colour of ventral side of nodes	3	red
011	Shoot: density of erect hairs on nodes	1	none or very low
012	Shoot: density of erect hairs on internodes	1	none or very low
013	Shoot: density of prostrate hairs on nodes	1	none or very low
014	Shoot: density of prostrate hairs on internodes	3	low
015-2	Shoot: intensity of anthocyanin coloration on bud scales	5	medium
016	Shoot: number of consecutive tendrils	1	2 or less
017	Shoot: length of tendrils	3	short
051	Young leaf: colour of the upper side of blade (4 th leaf)	4	copper-reddish
053	Young leaf: density of prostrate hairs between main veins on lower side of blade	3	low
054	Young leaf: density of erect hairs between main veins on lower side of blade	1	none or very low
055	Young leaf: density of prostrate hairs on main veins on lower side of blade	3	low
056	Young leaf: density of erect hairs on main veins on lower side of blade (4 th leaf)	1	none or very low
065	Mature leaf: size of blade	5	medium
067	Mature leaf: shape of blade	3	pentagonal
068	Mature leaf: number of lobes	3	five
069	Mature leaf: colour of the upper side of blade	5	medium green
070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1 bifurcate.
071	Mature leaf: area of anthocyanin coloration of main veins on lower side of blade	2	only at the petiolar
072	Mature leaf: goffering of blade	3	weak
073	Mature leaf: undulation of blade between main and lateral veins	1	absent
074	Mature leaf: profile of blade in cross section	5	twisted
075	Mature leaf: blistering of upper side of blade	3	weak
076	Mature leaf: shape of teeth	4	concave-convex
077	Mature leaf: size of teeth in relation to blade size	3	small
078	Mature leaf: length of teeth compared with their width	7	long
079	Mature leaf: degree of opening / overlapping of petiole sinus	5	closed
080	Mature leaf: shape of base of petiole sinus	3	V-shaped
081-1	Mature leaf: teeth in the petiole sinus	1	none
081-2	Mature leaf: petiole sinus base limited by veins	1	not delimited
082	Mature leaf: degree of opening / overlapping of upper lateral sinus	1	open
083-1	Mature leaf: shape of base of upper lateral sinuses	3	V-shaped
083-2	Mature leaf: teeth in the upper lateral sinuses	1	none
084	Mature leaf: density of prostrate hairs between the main veins on lower side of blade	3	low
085	Mature leaf: density of erect hairs between the main veins on lower side of blade	1	none or very low
086	Mature leaf: density of prostrate hairs on main veins on lower side of blade	3	low
087	Mature leaf: density of erect hairs on main veins on lower side of blade	1	none or very low

Table 1 (cont.). Mean values for the OIV (2009) ampelografic descriptors observed in Almuñécar cultivar during seven years (2003-2009)

OIV Code	Descriptor	Expression	Notes
088	Mature leaf: prostrate hairs on main veins on upper side of blade	1	absent
089	Mature leaf: erect hairs on main veins on upper side of blade	1	absent
090	Mature leaf: density of prostrate hairs on petiole	3	low
091	Mature leaf: density of erect hairs on petiole	1	none or very low
093	Mature leaf: length of petiole compared to length of middle vein	3	slightly shorter
094	Mature leaf: depth of upper lateral sinuses	5	medium
101	Woody shoot: cross section	2	elliptical
102	Woody shoot: structure of surface	3	striate
103	Woody shoot: main colour	2	brownish
104	Woody shoot: lenticels	1	absent
105	Woody shoot: erect hairs on nodes	1	absent
106	Woody shoot: erect hairs on internodes	1	absent
151	Flower: sexual organs	3	
152	Inflorescence: insertion of 1st inflorescence	2	3 and 4 node
153	Inflorescence: number of inflorescences per shoot	2	1,1 to 2
155	Shoot: fertility of basal buds (buds 1-3)	5	medium
202	Bunch: length (peduncle excluded)	7	long
203	Bunch: width	5	medium
204	Bunch: density	3	loose
206	Bunch: length of peduncle of primary bunch	3	short
207	Bunch: lignification of peduncle	1	at the base only
208	Bunch: shape	1	cylindrical
209	Bunch: number of wings of the primary bunch	2	1-2 wings
220	Berry: length	9	very long
221	Berry: width	5	medium
222	Berry: uniformity of size	1	not uniform
223	Berry: shape	4	narrow ellipsoid
225	Berry: colour of skin	1	green yellow
226	Berry: uniformity of colour of skin	1	not uniform
227	Berry: bloom	5	medium
228	Berry: thickness of skin	7	thick
229	Berry: hilum	2	visible
231	Berry: intensity of the anthocyanin coloration of flesh	1	none or very weak
232	Berry: juiciness of flesh	2	medium juicy
233	Berry: must yield	7	high
235	Berry: firmness of flesh	2	slightly firm
236	Berry: particularity of flavour	2	muscat
238	Berry: length of pedicel	3	short
240	Berry: ease of detachment from pedicel	3	difficult
241	Berry: formation of seeds	1	complete
242	Berry: length of seeds	7	long
243	Berry: weight of seeds	5	medium
244	Berry: transversal ridges on dorsal side of seeds	1	absent
301	Time of bud burst	5	medium
302	Time of full bloom	5	medium

Table 1 (cont.). Mean values for the OIV (2009) ampelografic descriptors observed in Almuñécar cultivar during seven years (2003-2009)

OIV Code	Descriptor	Expression	Notes
303	Time of beginning of berry ripening (veraison)	5	medium
304	Time of physiological stage of full maturity of the berry	5	medium
305	Time of beginning of wood maturity	5	medium
306	Time of autumn colouring of leaves	1	yellow
351	Vigour of shoot growth	5	medium
352	Growth of axillary shoots	5	medium
353	Length of internodes	3	short
354	Diameter of internodes	5	medium
452	Leaf: degree of resistance to Plasmopara	5	medium
452-1	Leaf: degree of resistance to Plasmopara (leaf disc test)	7	high
453	Cluster: degree of resistance to Plasmopara	5	medium
455	Leaf: degree of resistance to Oidium	5	medium
455-1	Leaf: degree of resistance to Oidium (leaf disc test)	7	high
456	Cluster: degree of resistance to Oidium	7	high
458	Leaf: degree of resistance to Botrytis	5	medium
458-1	Leaf: degree of resistance to Botrytis (laboratory analysis)	5	medium
459	Cluster: degree of resistance to Botrytis	5	medium

Table 2. Genetic profile of Almuñécar cultivar at 20 microsatellite loci. Allele sizes in base pairs

Microsatellite loci	Allele sizes	
VV1B01	291	295
VMC1b11	166	184
VMC4F3-1	182	206
VVMD5	226	228
VVMD7	246	248
VVMD21	255	265
VVMD24	213	213
VVMD25	246	246
VVMD27	180	194
VVMD28	246	270
VVMD32	262	270
VVIH54	166	166
VVIN16	149	151
VVIN73	264	264
VVIP31	188	192
VVIP60	318	322
VVIQ52	83	83
VVS2	131	149
VVIV37	163	175
VVIV67	375	389

Due to phylloxera, infestation, detected between 1880 and 1883 and droughts, several vineyards were abandoned in Almuñécar (Granada). In 1909 about 3,000 people living in the surrounding Almuñécar emigrated to Ledesma (Jujuy province, Argentine). The decline of raisin trading and the introduction of subtropical cultivars in the area and the high susceptibility of the Almuñécar cultivar to powdery and downy mildews reduced more its presence near extinction at present time.

This research has allowed the recovery of this ancient cultivar, that constitute a clone of Muscat of Alexandria, and its preservation in Rancho de la Merced germplasm bank (Jerez de la Frontera, Spain), where it constitutes a material to be used in further studies or for the establishmet of new plantations. Accurate identification of accessions is a basic requirement for the rational management and use of germplam.

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