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SHORT COMMUNICATION

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Identification and relationship of the autochthonous 'Romé' and 'Rome Tinto' grapevine cultivars

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Abstract

The 'Romé' variety is considered an Andalusian (southern region in Spain) autochthonous black grape cultivar. However, several white and black grapevine accessions are known by this name, according to *Vitis* International Variety Catalogue. The aim of the present work was to clarify the identity of the 'Romé' and 'Rome Tinto' as black grapevine cultivar. Eight accessions known as 'Romé' and two as 'Rome Tinto' were analyzed using 30 OIV descriptors and 22 SSR loci. The morphologic and genetic analysis showed that all accessions studied presented the same genotype and phenotype and grouped with South Spanish cultivars. This study helps to clarify the confusion over the identity of 'Romé' grapevine cultivar, and provides a solid basis to develop a germplasm collection to protect grapevine diversity and to recover cultivars that may be in danger of extinction.

Additional keywords: *Vitis vinifera*; SSR; ampelography; synonym.

Abbreviations used: SSR (Sample Sequence Repeats); VIVC (*Vitis* International Variety Catalogue).

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Introduction

Grapevine (*Vitis vinifera* L.) is a species that presents a wide genetic diversity and is widely conserved in germplasm banks. The existence of synonyms, homonyms and misnomers are one of the major problems for viticulture worldwide (Veloso *et al.*, 2010) and are obstacles for an international network on the conservation of *Vitis* germplasm in Europe (Maul, 2008). In order to clarify the misidentification and confusion in grapevine variety designations caused by the morphological characterization subjectivity, many collections have already been characterized using Sample Sequence Repeats (SSR) markers (Lopes *et al.*, 1999; Martín *et al.*, 2003, 2011; Ortiz *et al.*, 2004; De Mattia *et al.*, 2007; Dzhambazova *et al.*, 2009; Ibáñez *et al.*, 2009; Vargas *et al.*, 2009; Cipriani *et al.*, 2010; Laucou *et al.*, 2011; Lacombe *et al.*, 2013; Milla-Tapia *et al.*, 2013; Aliquó *et al.*, 2017). Although, this problematic is collected also in The *Vitis* International Variety Catalogue (VIVC, www.vivc.de), which is currently the source of reference to help group the varieties under a common and consensual identifying label (Lacombe *et al.*, 2011). A clear example of mistakes is registered for 'Romé' cultivar. The VIVC database (*Vitis* International Variety Catalogue [VIVC, www.vivc.de]) includes two varieties of Spanish origin called 'Rome': a white-berry cultivar (VICV 16035), which only is conserved in two Spanish collections [Finca El Encín (Institute Code VIVC ESP080) and Rancho de la Merced Germplasm Bank (Institute Code VIVC ESP074)], and a black-berry cultivar (VIVC 10181), whose conservation location is unknown. This European database also includes a variety with the prime name 'Rome Tinto' (VIVC 40905) that only is conserved in the Rancho de la Merced and its origin is uncertain.

Ibáñez *et al.* (2009) characterized a 'Rome' conserved in the Finca El Encín using 20 SSR loci. Results showed identical genotype with 'Muscat of Alexandria'. They proposed that it could be an unknown synonym for this cultivar. Similar results were obtained by García de Luján *et al.* (1990) with 128 OIV ampelographic descriptors by 'Rome' conserved in the Rancho de la

Merced collection. However, the genotype of 'Rome Tinto' is now unpublished.

The main objective of this work was to identify different 'Romé' and 'Rome Tinto' grapevine accessions that are conserved in the Rancho de la Merced Germplasm Bank with difference origin. Their genetic and morphologic characterization could help to detect synonyms, homonyms and false attributions. This research is necessary to provide a solid basis to develop a germplasm collection to protect grapevine diversity and to recover cultivars that may be in danger of extinction.

Material and methods

A total of eight accessions, six 'Romé' and two 'Rome Tinto', were analyzed using 22 SSR loci. Six of the 'Romé' accessions were collected during different prospecting trips carried out from 1999-2001 in vineyards situated at the localities of Cómpeta, Torrox and Ronda (province of Málaga, Andalusia community, South Spain). All accessions analyzed are conserved in the Rancho de la Merced Germplasm Bank. Furthermore, four reference cultivars ('Cabernet Sauvignon', 'Chardonnay', 'Muscat a Petits Grains Blancs' and 'Pinot Noir') were also included to test for genetic profiles obtained with the different databases published. In order to identify the geographical origin of this 'Romé' accessions, the genetic analyses were complemented with other cultivars sampled at the Rancho de la Merced Germplasm Bank that originally came from different geographical areas and included *Vitis vinifera* varieties ('Airén', 'Flame Seedless', 'Garrido Fino', 'Graciano', 'Mantúo de Pilas', 'Michele Palieri', 'Muscat of Alexandria', 'Muscat Hamburg', 'Ohanes', 'Palomino Fino', 'Pedro Ximenes', 'Syrah', 'Tempranillo', 'Vijiriega Común', 'Viura' and 'Zalema' 'Cabernet Sauvignon', 'Chardonnay', 'Muscat a Petits Grains Blancs' and 'Pinot Noir') and interspecific hybrids ('RM2' and 'Jacquez'), that were used as an outgroup in the genetic relationship analysis.

DNA extraction from plant material was performed using young leaves collected in spring. Genomic DNAs were isolated from 100 mg of frozen leaf tissue using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). A total 22 SSR loci were analysed in order to verify the varietal identity. A first set of 20 microsatellite loci located in the 19 linkage groups of grapevine genome [VMC1B11 (Zyprian & Topfer, 2005), VMC4F3-1 (Di Gaspero *et al.*, 2000); 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)] were analysed using two multiplex PCRs as described in a previous study (Vargas *et al.*, 2007). Another set of 2 microsatellite loci [VrZAG62 and VrZAG79 (Sefc *et al.*, 1999)] were analysed to complete the list of loci authorized by the International Organisation of Vine and Wine (OIV, 2009). These last two loci were used under the conditions detailed in a previous work (Jiménez-Cantizano *et al.*, 2006).

PCR amplifications were carried out in an Applied Biosystems 9700 thermocycler. Amplified products were separated by capillary electrophoresis using an automated sequencer (ABI Prism 3130, Appl. Biosyst.). Fluorescently labelled fragments were detected and sized using GeneMapper v. 3.7 software (Appl. Biosyst.) and fragment lengths were determined with the help of internal size standards (GeneScan-500 LIZTM, Appl. Biosyst.). SSR profile comparisons were carried out using the Microsatellite toolkit v. 9.0 software package (Park, 2001). Genetic distances between grapevine genotypes were calculated as [-ln (proportion shared alleles)] using Microsat (Minch *et al.*, 1997). The obtained data were used for the construction of a dendrogram using the program EXE from the PHYLIP software package (Felsenstein, 1989) and Treeview (Page, 1996).

Ampelographic analyses were carried out during three years (2012-2015) and using the descriptor list for grapevine cultivars and *Vitis* species from the OIV (2009) using 5 plants per accession.

Results and discussion

All accessions 'Romé' and 'Rome Tinto' characterized showed identical genotype at 22 SSR loci (Table 1). The genotype obtained was not included in published databases (Ibáñez *et al.*, 2009; Vargas *et al.*, 2009; De Andrés *et al.*, 2012; Lacombe *et al.*, 2013; Jiménez-Cantizano, 2014; and VIVC (www.vivc.de)), thus, this new genotype corresponds to a new grape cultivar. According to the results obtained in this study, the 'Rome Tinto' accession registered in the VIVC database with the variety number "40905", should be considered a synonym of 'Rome' "10181", which could be the true-to-type of the variety prime name. This variety is conserved only in the Rancho de la Merced Germplasm Bank. On the other hand, morphological characterization showed the same phenotypes in all 'Romé' and 'Rome Tinto' accessions (Table 2). This variety could be 'Romé' (black-berry cultivar) described in Andalusia by several authors in the 16th (Herrera, 1513) and 19th century (Clemente-Rubio,

Table 1. Genetic profile of 'Rome' accessions, reference varieties and other cultivars analyzed at 22 SSR loci. Allele sizes are given in base pairs.

Database Code ESP074-xxx	Accession name	VVIB01		VM-C1b11		VM-C4F31		VVMD5		VVMD7		VVMD21		VVMD24		VVMD25		VVMD27		VVMD28		VVMD32		VVIH54	
626	Romé	291	295	184	188	188	190	236	238	236	236	243	249	209	209	240	252	182	194	238	260	254	270	166	166
627	Romé	291	295	184	188	188	190	236	238	236	236	243	249	209	209	240	252	182	194	238	260	254	270	166	166
628	Romé	291	295	184	188	188	190	236	238	236	236	243	249	209	209	240	252	182	194	238	260	254	270	166	166
629	Romé	291	295	184	188	188	190	236	238	236	236	243	249	209	209	240	252	182	194	238	260	254	270	166	166
630	Romé	291	295	184	188	188	190	236	238	236	236	243	249	209	209	240	252	182	194	238	260	254	270	166	166
631	Romé	291	295	184	188	188	190	236	238	236	236	243	249	209	209	240	252	182	194	238	260	254	270	166	166
632	Rome Tinto	291	295	184	188	188	190	236	238	236	236	243	249	209	209	240	252	182	194	238	260	254	270	166	166
633	Rome Tinto	291	295	184	188	188	190	236	238	236	236	243	249	209	209	240	252	182	194	238	260	254	270	166	166
REFERENCE CULTIVAR																									
Cabernet Sauvignon		291	291	184	184	174	178	228	238	236	236	249	257	209	217	238	246	176	190	236	238	238	238	166	182
Chardonnay		289	295	166	184	174	180	232	236	236	240	249	249	209	217	238	252	182	190	220	230	238	270	164	168
Muscat a Petits Grains Blancs		291	295	184	188	168	206	226	324	323	246	249	265	213	217	240	246	180	194	248	270	262	270	166	166
Pinot Noir		289	295	166	172	174	180	226	236	236	240	249	249	215	217	238	246	186	190	220	238	238	270	164	168
OTHER CULTIVARS[1]																									
Airén		291	291	184	184	174	188	224	232	240	250	243	265	209	213	252	252	182	194	246	246	250	270	166	166
Flame seedless		291	295	166	166	168	168	232	234	236	250	249	255	209	209	240	246	182	186	246	246	248	270	166	166
Garrido fino		291	307	184	188	174	204	226	234	236	246	249	265	211	213	240	240	194	194	246	260	260	270	166	168
Graciano		291	291	172	184	180	206	224	236	236	236	249	253	209	209	262	268	180	184	246	260	238	254	168	168
Jacquez		289	291	178	184	184	184	226	240	236	238	237	249	209	217	254	256	180	190	232	238	250	250	166	166
Mantuo de Pilas		307	307	184	188	184	190	224	232	244	246	243	249	209	209	238	252	182	182	246	248	270	270	166	168
Micheli de Palieri		291	295	166	186	168	168	236	236	240	252	249	249	209	211	252	252	186	194	236	246	250	270	166	168
Muscat of Alexandria		291	295	166	184	182	206	226	228	246	248	255	265	213	213	246	246	180	194	246	270	262	270	166	166
Muscat Hamburg		295	295	166	172	174	206	228	236	244	246	249	255	213	213	246	252	180	186	238	246	270	270	166	166
Ohanes		291	295	184	188	188	190	232	234	232	248	255	255	209	209	252	252	184	194	246	250	260	270	166	166
Palomino Fino		291	307	184	188	176	206	226	238	236	246	243	249	209	209	240	240	186	194	238	250	254	256	166	166
Pedro Ximenes		291	307	168	188	168	174	234	238	236	236	243	249	209	213	240	246	182	186	260	266	248	270	166	166
RM2		291	297	184	188	174	174	228	262	230	262	249	249	203	213	248	248	190	190	238	254	238	238	144	144
Syrah		291	295	166	188	174	206	224	228	236	236	247	265	209	215	240	240	190	192	220	230	238	270	164	166
Tempranillo		291	295	172	184	180	184	234	234	236	250	247	255	209	215	240	252	184	184	260	260	248	250	164	166
Vijiriega común		291	291	172	188	168	188	232	236	236	246	249	249	209	211	240	252	182	186	260	260	254	270	166	168
Viura		291	295	184	184	180	188	232	234	236	236	243	253	209	211	238	240	190	194	238	260	248	254	166	166
Zalema		291	307	188	188	174	188	234	238	236	236	249	255	209	211	240	240	182	182	250	250	254	270	166	168

1807; Abela & de Andino, 1885). In this way, 'Romé' (white-berry) characterized by García de Luján *et al.* (1990) and Ibáñez *et al.* (2009) identified as 'Muscat of Alexandria' could be a misnamed.

The resulting dendrogram using the UPGMA method (Fig. 1) defines the genomic relationships among the analyzed cultivars and shows the existence of six groups, denoted A-F. The formation of these groups may be related to the use of the cultivars and regions of origin. According to the results, 'Romé' cultivar is grouped (Group A) with white cultivars ('Zalema', 'Pedro Ximenes', 'Viura', 'Palomino Fino' and 'Garrido Fino') that are of Spanish origin and used in winemaking. Within this group A 'Romé' has a greater affinity with 'Zalema', 'Pedro Ximenez' and 'Viura' (Fig. 1). These three varieties share first-degree

Table 1. Continued.

Database Code ESP074-xxx	Accession name	VVIN16		VVIN73		VVIP31		VVIP60		VVIQ52		VVS2		VVIV37		VVIV67		VrZAG62		VrZAG79	
626	Romé	153	153	264	264	176	190	318	326	83	89	135	144	163	171	366	375	187	195	246	256
627	Romé	153	153	264	264	176	190	318	326	83	89	135	144	163	171	366	375	187	195	246	256
628	Romé	153	153	264	264	176	190	318	326	83	89	135	144	163	171	366	375	187	195	246	256
629	Romé	153	153	264	264	176	190	318	326	83	89	135	144	163	171	366	375	187	195	246	256
630	Romé	153	153	264	264	176	190	318	326	83	89	135	144	163	171	366	375	187	195	246	256
631	Romé	153	153	264	264	176	190	318	326	83	89	135	144	163	171	366	375	187	195	246	256
632	Rome Tinto	153	153	264	264	176	190	318	326	83	89	135	144	163	171	366	375	187	195	246	256
633	Rome Tinto	153	153	264	264	176	190	318	326	83	89	135	144	163	171	366	375	187	195	246	256
REFERENCE CULTIVAR																					
Cabernet Sauvignon		153	153	264	268	188	188	306	314	83	89	137	151	163	163	364	372	187	193	246	246
Chardonnay		151	151	264	266	180	184	318	322	83	89	135	142	153	163	364	372	187	195	242	244
Muscat a Petits Grains Blancs		149	149	264	264	184	188	318	318	83	83	131	131	163	165	364	375	185	195	250	254
Pinot Noir		151	159	264	266	180	180	318	320	89	89	135	151	153	163	364	372	187	193	238	244
OTHER CULTIVARS[1]																					
Airén		151	153	264	264	190	190	322	322	85	89	142	144	161	163	352	366	187	199	246	258
Flame seedless		153	157	262	264	184	190	318	318	85	89	131	151	151	181	358	372	187	187	246	250
Garrido fino		153	153	264	264	190	190	314	322	85	87	131	131	163	163	366	375	193	195	256	258
Graciano		151	159	264	264	180	192	312	318	89	89	137	151	165	177	358	364	184	187	250	258
Jacquez		149	151	262	270	176	182	316	318	85	85	137	142	159	171	334	364	185	197	248	248
Mantuo de Pilas		151	153	264	264	176	190	318	326	85	89	131	142	161	161	372	375	187	193	242	248
Micheli de Palieri		151	153	264	264	188	190	322	322	83	89	133	135	163	163	372	375	187	203	250	256
Muscat of Alexandria		149	151	264	264	188	192	318	322	83	83	131	149	163	175	375	375	184	203	246	254
Muscat Hamburg		151	157	264	264	180	188	318	322	83	87	133	149	163	171	372	372	184	191	254	254
Ohanes		149	153	264	264	180	190	318	326	85	89	131	135	153	161	358	362	199	203	250	256
Palomino Fino		151	151	256	264	188	190	318	322	85	85	131	144	163	167	364	366	187	193	250	256
Pedro Ximenes		149	153	264	264	176	188	322	322	89	89	131	144	163	177	364	366	187	187	242	246
RM2		149	153	254	262	184	184	312	328	83	85	146	149	151	161	372	372	ND	ND	ND	ND
Syrah		151	153	264	264	182	190	318	318	89	89	131	131	163	165	362	382	187	193	244	250
Tempranillo		151	153	256	256	180	180	326	326	85	85	142	144	171	171	366	368	195	199	246	250
Vijiriega común		149	153	264	264	184	190	322	322	85	89	135	144	153	163	358	372	187	203	246	250
Viura		153	153	264	264	176	196	318	326	85	89	131	144	161	161	372	375	187	187	242	256
Zalema		153	153	264	264	176	188	318	326	85	85	131	144	163	177	364	372	187	195	246	256

ND: Not determined. [1] Cultivars were used in the genetic relationship analysis.

relationships with 'Hebén' (Lacombe *et al.*, 2013; Zinelabidine *et al.*, 2015). In addition, 'Romé' presented the same genotype as the 'Viura' cultivar for seven SSR loci (Table 1): VVIB01, VVMD7, VVMD28, VVIH54, VVIN16, VVIN73 and VVIP60. These results could indicate that 'Romé' variety have 'Hebén' as a female parent. 'Hebén' is a variety, already described in the 16th century (Herrera, 1513) as a white variety of grapevine and it was grown in the Andalusian region (García de los Salmones, 1914). The white female 'Hebén' proved to be a key genitor in the Iberian Peninsula (Lacombe *et al.*, 2013; Zinelabidine *et al.*, 2015). This variety seems to originate from North Africa (Galet, 2000), which would be consistent with the relationships between Spanish

Table 2. Mean values for the OIV (2009) ampelografic and morphologic descriptors observed during three years (2012-2015).

OIV Code	Romé (ESP074-626, ESP074-627, ESP074-628, ESP074-629, ESP074-630, ESP074-631)	Rome Tinto (ESP074-632, ESP074-633)	Airén	Flame Seedless	Garrido Fino	Graciano	Jacquez	Mantúo de Pilas	Micheli de Palieri	Muscat of Alexandria	Muscat Hamburg	Ohanes	Palomino Fino	Pedro Ximenes	Syrah	Tempranillo	Vijiriega común	Viura	Zalema	Cabernet Sauvignon	Chardonnay	Muscat a Petits Grains Blancs	Pinot Noir	RM2[1]
001	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3
016	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
065	5	5	7	7	5	7	5	5	7	5	7	7	7	7	5/7	7	7	5/7	7	5	5	5	3	3
067	4	4	3	2	3	3	3	3	3	3	3	2	3	3	3	3	2	3	2	4	3	3	2	2
068	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	3	2	1
070	1	1	1	4	1	3	1	1	1	3	1	1	3	1	1	1	3	1	3	1	1	1	1	1
072	5	5	1	1	3	1	3/5	5	5	3	3	5	5	1	5	5	3	5	3	3	3	5	3	3
076	3	3	2	5	2	2	3	2	3	2	5	2	2	5	3	2	2	5	2	3	2	2	3	2
079	7	7	3	3	3	7	5	3	7	7	3	3	3	3	3	5	3	3	3	7	3	5	3	-
080	2	2	2	3	3	3	3	2	3	3	3	3	3	3	3	3	3	2	3	1	3	3	3	2
081-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
081-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	3
082	4	4	1	3	1	1	1	4	3	1	1	1	1	1	4	3	1	4	1	3	1	3	1	5
083-1	1	1	2	3	3	2	1	3	3	3	3	3	2	3	3	2	3	3	3	1	3	3	3	-
083-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1
084	7	7	9	1	5	7	3	5	3	3	1	1	7	1	5	7	1	5	1	3	1	1	1	1
085	7	7	7	1	1	7	3	5	1	5	1	1	7	5	3	7	1	1	1	5	3	1	1	3
202	7	7	7	3	5	3	3	7	7	7	7	5	7	7	5	7	3	7	3	3	3	5	3	1
204	5/7	5/7	5	5	5	7	3	5	5	3	5	3	5	3	5	9	7	7	7	5/7	5/7	7	7	3
220	5	5	5	5	5	3	3	5	7	7	7	7	3	5	3/5	5	5	5	5	3	3	5	3	1
223	2	2	3	3	2	3	2	2	6	4	3	5	2	3	3	2	3	2	3	2	2	2	2	2
225	3	3	1	3	1	6	6	1	5	1	5	1	1	1	6	6	1	1	1	6	1	1	6	6
235	1	1	2	3	2	1	1	2	2	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1
236	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	4	1	2	1	1
241	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
502	5	5	7	5	5	3	5	5	7	5	9	5	7	5	5	7	5	5	5	3	3	5	3	1
503	3	3	5	3	3	5	5	3	7	5	7	5	3	3	5	3	3	7	3	3	3	3	3	1
505	1	1	1	1	5	1	5	3	1	1	1	1	1	3	5	1	1	7	1	5	5	5	5	-
506	3	3	3	3	3	3	7	5	3	3	3	3	3	3	5	3	3	3	3	3	3	3	5	-
508	3	3	5	7	7	5	3	5	7	5	5	5	5	5	5	5	5	3	5	5	5	5	3	-

[1] RM2: Rootstock.

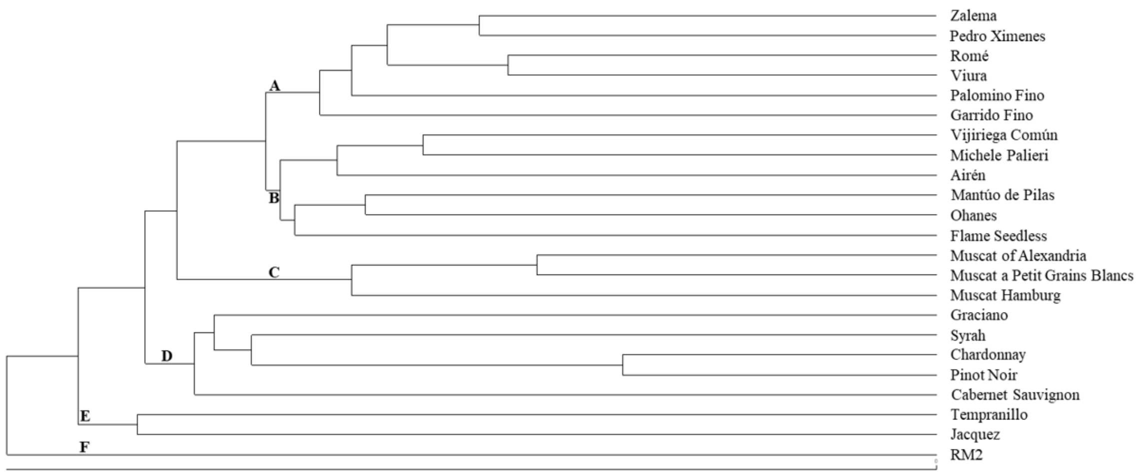

Figure 1. Dendrogram representing relationships among the different studied cultivars, based on molecular data using UPGMA as grouping method. A-F, formed groups; see text for comments.

and North African grape gene pools (El Oualkadi *et al.*, 2011). In grapevine numerous changes have occurred as a result of human selection, including the emergence of hermaphroditism and greatly increased variation in berry color (This *et al.*, 2007). Previous research works showed that approximately 800 bp insertion is present in a few red-skinned varieties that are somatic mutants of white-skinned varieties (Kobayashi *et al.*, 2004). In addition, according to Lijavetzky *et al.* (2006) *VvmybA1* gene could be a major determinant of berry colour variation in grapes. The results obtained in a study of the examined allelic variation in *VvmybA1* in over 200 accessions of cultivated grapevine including several well characterized fruit color mutants, indicated that the white fruited-allele of *VvmybA1* most likely arose a limited number of times and that variation in this gene is likely responsible for the majority of the fruit color variation present in modern grapevine cultivars (This *et al.*, 2007). In this sense, this same effect may have occurred with the 'Romé' variety.

Autochthonous cultivars are a genetic resource which could play an important role in the future, to study their warm climatic conditions adaptation capacity and their oenological potential into new wines. According to Fraga *et al.* (2016), adapting to future climates may comprise a selection of more resilient varieties to warming and drying. In this regard, studies carried out with Portuguese varieties (Lopes *et al.*, 2008; Fraga *et al.*, 2016) shows that genetic, morphological and physiological differences between each variety allows a better adaptation to different climates resulting in of singular wines production. According to the EU and Spanish normative only registered plant material is possible to be used in new plantations. As a conclusion, 'Romé' cultivar could be considered as an Andalusian autochthonous black grape cultivar and its correct identification could facilitate 'Romé' inclusion in the Official Register of Spanish grapevine varieties.

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