

Research Note

Looking for old grapevine varieties

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Introduction: The Comunitat Valenciana is an important viticulture area since historic times and its richness in grapevine varieties is well documented (DÍES-CUSÍ *et al.* 1993; suppl. data S1). In 1889, the black grape cultivars 'Bobal', 'Gateta', 'Garnacha', 'Monastrell', 'Morenillo', 'Pampolat', 'Planta de Mula', 'Tintorero Hybrid' and 'Valenci negre'; the table red grapevines 'Roget de Chella', 'Botó de Gall', 'Corinto rojo', 'Planta de Elda', 'Rojal' and 'Roget de vinya'; and the white grapevines 'Albillo', 'Grec', 'Jaén', 'Macamen', 'Merseguera', 'Moscatell romà', 'Palop' (syn. 'Calop' or 'Valenci'), 'Planta d'Engord', 'Planta nova', 'Trepadell' and 'Vidriell' occupied the higher amount of cultivated areas (DGAIC 1891). Phylloxera arrived in 1905–1906 extending to the high-yielding areas in 1912 and, as a consequence, a great genetic erosion was produced. Fortunately, some of the most ancient grapevine varieties present in Spain are preserved in the germplasm collection 'El Encín' (IMIDRA, Madrid). Different actions aiming at recovering of ancient Spanish grapevine varieties have been reported (FERNÁNDEZ-GONZÁLEZ *et al.* 2007, GARCÍA-MUÑOZ *et al.* 2012, MARTÍN *et al.* 2003, URRESTARAZU *et al.* 2015, MARSAL *et al.* 2017). However, the Comunitat Valenciana was scarcely represented in these works. In the present work, we report on the localization and SSR profiles of varieties surveyed in old vineyards (some of them not described in databases), the synonymies found, the chlorotypes assigned as well as new pedigree relationships.

Material and Methods: Suppl. data S2 shows information (name and location) about the 115 assessed accessions as well as the provenance of the eight samples included as controls. DNA extractions were performed using the commercial DNeasy Plant Mini Kit (Qiagen). Fifteen nuclear SSR markers (VVS2, VVMD5, VVMD6, VVMD7,

VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG64, VrZAG79, VrZAG83, and VMC1b11) were analyzed using two sets of multiplex PCR reactions as described in PEIRÓ *et al.* (2018). Chlorotypes were determined in new accessions as well as in those accessions without information in the *VIVC* database. Five SSRs (CCMP3, CCMP5, CCMP10, ccSSR9 and ccSSR14) as described by ARROYO-GARCÍA *et al.* (2006) were used.

Results and Discussion: The SSR profiles (suppl. data S3) confirmed that 104 analyzed accessions corresponded to varieties that were present in the surveyed area in the pre-phylloxera era (suppl. data S1). On the other hand, six varieties surveyed with names of ancient cultivars did not match with any accession in the *VIVC* database: 'Arcos' (also collected as 'Arco Forcallà'), 'Macabeo negro', 'Mamella de Vaca', 'Montalbana', 'Raïm del Clotet' and 'Trepadell'. However, with the name of 'Arcos' in this database, appeared two accessions which correspond to 'Moristel' (variety 12353) and 'Miguel de Arco' (variety 24602). Other three accessions, two surveyed as 'Malvasia' and another unnamed showed unique profiles and still remain unknown. In addition, we found that all the accessions surveyed as 'Botó de Gall' had the same profile than 'Ahmeur bou Ahmeur' (variety 140 in *VIVC*), a classical cultivar of Argelia. Therefore, both names are synonyms. Similarly, it is the first time that the variety 'Morsí' (mentioned in the pre-phylloxera documents) is reported in Spain and resulted to be a synonym to 'Sbaa Tolba' (variety 10804 in *VIVC*). Interchange of grapevine cultivars between the North of Africa and Spain from 711 to 1492 AD was common. Of great interest was also the localization of seven accessions with the same SSR profile as 'Muscat d'Istambul' (variety 17493 in *VIVC*), four of them collected as 'Grumer Moscatell' and another three under the names of 'Moscatel del Terreno', 'Moscatel Dulce' and 'Moscatel Alicante'. Previously, we identified this variety with the names of 'Gustico de Elche', 'Grumer Moscatell' and 'Moscatell d'Alfàbega' (PEIRÓ *et al.* 2018). In FAVÀ-AGUD (2001), there is a reference to 'Grumer muscatel' under the entry 'Grumer'. Also the name 'Moscatel de Elche' was mentioned in a report of 1877. LACOMBE *et al.* (2013) proposed as the origin of 'Muscat d'Istambul' the crossing of 'Muscat of Alexandria' x 'Valenci blanc' which was confirmed by MENA *et al.* (2014) and fits with our 15 SSRs genotyping. Taking into account the results of chlorotypes for these accessions, 'Valenci blanc' (also named 'Grumer') will be the mother of the cross. Therefore, we think that the most appropriate name for this variety would be 'Grumer Moscatell'. In addition, the same profile than 'Marufo' (variety 8086 in *VIVC*) was found for the accession of 'Crujidera', for four unknown accessions, and for two accessions surveyed in Ademuz (Valencia) under the name of 'Camera'. Therefore, this latter is a local synonym of 'Crujidera' that is synonym of 'Marufo'. Despite the fact that 'Marufo' and

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Table

New profiles, synonymies, and/or chlorotype in grapevine accessions

Cultivar name ¹⁾	Identification name ²⁾	Chlorotype
Arco Forcallà/Arcos	<i>Arcos</i>	A
Macabeo negro	<i>Macabeo negro</i>	D
Mamella de Vaca	<i>Mamella de Vaca</i>	D
Montalbana	<i>Montalbana</i>	D
Raïm del Clotet	<i>Raïm del Clotet</i>	C
Trepadell	<i>Trepadell</i>	A
Malvasia 3	<i>NI-1</i>	D
Malvasia 5	<i>NI-2</i>	A
U25	<i>NI-3</i>	B
Botó de Gall	<u>Ahmeur bou Ahmeur</u>	C
Mondragón	Mondragón	D
Morenillo	Morenillo	A
Morsí	<u>Sbaa Tolba</u>	C
Planta Mula	Planta Mula	D
Benicarló	Benicarló	A
Esclafagerres	Esclafagerres	A
Gateta	Gateta	D
Pampolat	Pampolat de Sagunt	A

¹⁾ Name given by grower.

²⁾ The SSRs profiles were compared with those in *VIVC* (2019). Names in italics refer to new profiles firstly reported in this work; underlined names refer to new synonymies.

'Moravia Dulce' are considered synonyms in some reports, *VIVC* proposes the crossing of 'Marufo' x 'Valenci blanc' as pedigree of 'Moravia Dulce' (variety 23166 in *VIVC*). This pedigree was also proposed by MENA *et al.* (2014) for 'Valenci negro'. However, this latter is not supported by our genotyping, due to the incompatibility found at three loci (VVMD27, VVMD28, and VrZAG64). Recently, the pedigree of 'Mondragón' ('Monastrell' x 'Marufo') was reported (LAUCOU *et al.* 2018). Taking into account the results of our chlorotype analysis, 'Marufo' was the female parent in this cross. We also found that this cross is compatible with the pedigree of the varieties 'Macabeo negro' and 'Montalbana'. On the other hand, it was found that 'Valenci blanc' can be putatively the parent of 'Tortosina' ('Tortosi') and 'Planta Fina' can be involved with the pedigree of 'Trepadell'.

This work confirms that an important biodiversity reservoir still remains at the Comunitat Valenciana. Among 115 analyzed accessions, unique SSR profiles and unique alleles were found. Besides, with this work we also contributed to assign chlorotypes, detect synonymies as well as suggest new relationships.

We are performing different actions which include *in situ* and *ex situ* conservation of this local germplasm. Plants of 'Mondragón', 'Montalbana' or 'Raïm del Clotet' are really scarce but are maintained in the Rebollar collection (GVA) and propagating material was sent to 'El Encin' as well as to 'The Domain de Vassal' repositories. In order to preserve and characterize germplasm, propagating material of all identified genotypes has been donated to some nurseries and/or wineries to establish private collections and have

complementary data on ampelographic characteristics and vinification aptitude. Another collection has been set up in the Botanical Garden of the University of Valencia.

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