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Recovering Ancient Grapevines Varieties in the Spanish provinces of Alicante and Valencia

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Abstract

The provinces of Alicante and Valencia, located in the Mediterranean coast of Spain, are important viticulture areas since historic times. In the context of the research project CGL2015-70843-R, we initiated different approaches in order to recover ancient varieties threatened of disappearance and we developed *in vitro* protocols for virus sanitation and in vitro conservation of the recovered germplasm. Among the historic varieties, we localized several accessions of varieties that were commonly grown in the pre-phylloxera era in the provinces of Alicante and Valencia (i.e. 'Valencí blanc', 'Valencí negre', 'Planta Mula', 'Botó de Gall' or 'Raïm del Clotet'). Microsatellite profiles were obtained to confirm or identify the surveyed germplasms and genetic variability was observed. In addition, a survey was carried out covering the main area of 'Monastrell' cultivation in the Vinos de Alicante Protected Designation of Origin. This ancient variety, also known as 'Mourvèdre', is cultivated mainly in the southeast of Spain and it is highly adapted to the dry and warm climate of this area. Genotyping by Sequencing (GBS) was used to estimate the genetic diversity of this variety and high variability was found. This analysis will provide a high number of high quality SNPs well distributed across the genome suitable for genotyping of clones, which will allow the design of strategies optimizing their conservation and use.

Keywords: 'Botó de Gall', 'Monastrell', 'Planta Mula', 'Raïm del Clotet', 'Valencí blanc', 'Valencí negre'

INTRODUCTION

The provinces of Alicante and Valencia are located in the Mediterranean coast of Spain and are important viticulture areas since ancient times. At the archeological place 'Alt de Benimaquía' different evidences (wine presses, seeds and residues of vinification and pottery) indicated that wine was already produced at the VII century BC. Nowadays, three Protected Designations of Origin (PDO) for wine production (PDO Vinos de Alicante, PDO

Valencia and PDO Utiel-Requena) and one for table grape (PDO Uva de mesa del Vinalopó) are present in these provinces.

Before the arrival of the phylloxera (*Daktulosphaira vitifoliae* Fitch) pest, more than 150 grapevine cultivars were cultivated in this area (DGAIC, 1891). Then, a strong genetic erosion occured. The creation of PDO, which permit the use of a small number of varieties, has also contributed to the reduction of ancient autochthonous varieties.

Despite the fact that several works have been conducted to recover ancient Spanish grapevine varieties (Fernández-González el al., 2007; García Muñoz et al., 2012; Marsal et al., 2017; Martín et al., 2003; Urrestarazu et al., 2015), the provinces of Alicante and Valencia are poorly represented. Therefore, in the research project CGL2015-70843-R (UPV), we initiated different approaches aiming to recover and conserve ancient varieties that are threatened of disappearance: prospections in ancient or neglected vineyards; identification of varieties by SSRs; analysis of grapevine germplasm variability and the development of protocols for virus sanitation and in vitro conservation (San Pedro et al., 2017; San Pedro et al., 2018; Gisbert et al., 2018; Peiró et al., 2018). On the other hand, at UMH, a survey was carried out to evaluate genetic variability in the autochthonous variety 'Monastrell', a variety adapted to dry and warm climate.

MATERIALS AND METHODS

SSRs profiles and analysis of variability

Grapevine accessions were surveyed in Alicante and Valencia provinces. Young leaves were used for DNA extractions, which were made using the commercial DNeasy Plant Mini Kit (Qiagen). PCR reactions which amplified with the following SSR markers (VVS2, VVMD5, VVMD6, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG64, VrZAG79, VrZAG83, and VMC1b11) were performed using two sets of multiplex as described in Peiró et al. (2018). PCR products were previsualized using gel electrophoresis carried out on an ABI 3100 platform (Applied Biosystems). SSRs profiles were compared with those in VIVC (Vitis International Catalogue) and/or in The European Vitis Database.

Forty six accessions of 'Monastrell' were surveyed in Alicante province in different old vineyards. GBS (Genotyping by Sequencing) was performed using the endonuclease ApeKI (G/CWGC) and the sequencing platform Illumina HiSeq 2000 (Illumina Inc.). Around 75% of reads (high quality reads) have been mapped to the reference genome developed by the French-Italian consortium (*V. vinifera* IGGP 12x).

Virus analysis

The presence of the viruses *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Grapevine fleck virus* (GFkV), *Grapevine leafroll associated virus-1* (GLRaV-1), and *Grapevine leafroll associated virus-3* (GLRaV-3) was evaluated. Leaf extractions were performed like in Peiró et al. (2015). The real-time multiplex RT-PCR was performed according to López-Fabuel et al. (2013) for the simultaneous detection of ArMV, GFLV, GFkV, GLRaV-1, and GLRaV-3 viruses. Amplifications were performed in a StepOne Plus thermocycler (Applied Biosystems).

RESULTS AND DISCUSSION

Prospection in old and neglected vineyards identified old grapevine varieties that were commonly grown in Alicante and Valencia provinces before the phylloxera attack (DGAIC, 1891). Among them, 'Valencí blanc', 'Valencí negre', 'Botó de Gall', 'Planta Mula', 'Raïm del Clotet' and several accessions of 'Muscat' were found. In the surveyed area, the variety 'Valencí blanc' as well as 'Muscat of Alexandria' are also conserved in particular orchards for table grape consumption; the rest of varieties are scarce, mainly 'Raïm del Clotet'. The SSR profiles of collected accessions were obtained and compared with those in the VIVC database (www.vivc.de): 'Valencí blanc', 'Valencí negre' and 'Planta Mula' had the same SSR profiles for comparable SSRs as the respective accessions confirming their identities. The profile of 'Botó

de Gall', matched that of the variety 'Ahmeur bou Ahmeur'. Indicating that both were synonyms. However, no reference was found either in this database or in other databases for 'Raïm del Clotet' which raisins showed a peculiar hole (Fig. 1). Concerning 'Muscat' germplasms, we found mainly 'Muscat of Alexandria' and a 'Muscat' that showed the same profile as the accession 17493 in the VIVC database, which corresponds to 'Muscat of Istambul'. This variety is reported to result from a cross between 'Valencí blanc' x 'Muscat of Alexandria' (Lacombe et al., 2013; Mena et al., 2014). We found this variety under the names of 'Grumer Moscatell', 'Gustico de Elche' and 'Moscatell d'Alfabega' (Peiró et al., 2018) and in posterior surveys, also as 'Moscatell del Terreno', 'Grumer Moscatell', 'Moscatel dulce' and 'Moscatel de Alicante' (Jiménez et al., under revision). We consider that the most appropriated name for this variety is 'Grumer Moscatell'. The name of 'Grumer' is common in old documents and a reference to 'Grumer Moscatell' was also found in a review of old grapevine names cultivated in the area where the survey had been carried out (Favà, 2001). Genetic variability was also found in 'Valencí blanc' (Gisbert et al., 2018), 'Muscat of Alexandria' and 'Grumer Moscatell' (Peiró et al., 2018). The SSR profiles of the endangered variety 'Esclafagerres,' previously localized by J. García and that of 'Raïm del Clotet' were first reported by Gisbert et al. (2018) and Jiménez et al. (under review), respectively.

Genotyping by Sequencing (GBS) was used in order to estimate the genetic diversity of the autochthonous variety 'Monastrell' (also known as 'Mourvèdre') surveyed in the Alicante province. A high number of high quality SNPs well distributed across the genome was obtained (ranging from 14,000 to 43,000 per sample, with coverage greater than 10X.). The validation of those polymorphic SNPs among 'Monastrell' clones will be suitable for clone genotyping and other association studies. A protocol for in vitro culture and multiplication was also previously developed for this variety (San Pedro et al., 2017) that has great importance in this area.

The EU Directive 2002/11/EC rules require that the initial plant material for vegetative propagation is free of ArMV, GFLV, GFkV, GLRaV-1 and GLRaV-3. In the surveyed material, the ArMV was not found in any sample whereas the rest of viruses are commonly found as occurred in other works (Bertolini et al., 2010); even double or triple infections were detected in 37 and 23% of the analyzed samples. Therefore, it is necessary to check the virus presence in the surveyed material and apply virus cleaning prior to in vitro storage.

Conclusions

In the Comunitat Valenciana old pre-phylloxera varieties threatened of disappearance can be found. Some of the germplasms identified were not reported previously in grapevine databases such as 'Raïm del Clotet' and 'Esclafagerres'. Genetic variability has been observed among accessions of different old grapevine varieties. High genetic variability was also found in the autochthonous variety 'Monastrell'. All these variability can be of interest in order to select genotypes with differential traits. Several synonymies were found for 'Grumer Moscatell' which we consider the most appropriate name. The meaning of some of these names indicates that this variety is appreciated by its sweetness. Given that several accessions of this variety were found in different old vineyard, it has been present in the surveyed area since old times.

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Figure 1. Grapes of the variety Raïm del Clotet