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Genetic variability in grapevine clones of 'Muscat of Alexandria'

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Abstract

Among grapevine varieties, the 'Muscat' family includes several widespread types that share a characteristic pronounced floral aroma and a typical 'Muscat' flavor. 'Muscat à Petit Grains blanc' and 'Muscat of Alexandria' are the most representative and ancient varieties. The grapevine variety 'Muscat of Alexandria' is of great importance within the 'Valencia' and 'Vinos de Alicante' PDO (Protected Designation of Origin, a prestigious Spanish regional product classification). Fruits from this variety are the basis of different appreciated wines, being also consumed as table grapes or used for raisin production. We used a set of selected SSR markers to confirm the identity of different clones of 'Muscat of Alexandria', some of them showing differential ampelographic traits. Additionally, we found intra-varietal genetic variability using AFLP markers. Now, a more accurate genotyping has been conducted using GBS (genotyping by sequencing). The GBS generated from 2 to 4 million of reads per sample, of which 85% were mapped to the reference genome developed by the French-Italian consortium (*V. vinifera* IGGP 12x). Around 40,000 SNPs were identified, with a coverage greater than 10X. Polymorphisms between and within the analyzed clones were found. The experimental validations of the identified SNPs will provide markers to accurately fingerprint these clones. They will be also suitable for association studies or to develop molecular markers useful in selection programs.

Keywords: SSRs, AFLPs, GBS, Comunitat Valenciana, La Marina Alta

INTRODUCTION

The 'Muscat' grapevines varieties display a characteristic pronounced floral aroma and a typical flavor ('Muscat' flavor) due to a great monoterpene concentration in their grapes (Mateo & Jimenez, 2000). The most representative varieties in the 'Muscat' family are 'Muscat à Petit Grains blanc' and 'Muscat of Alexandria' (Robinson, 1986). The latter is confirmed to be a natural crossing of 'Muscat à Petit Grains blanc' and a white-skinned table grape variety from Greek islands known as 'Axina de Tres Bias' or 'Heptakilo' (Cipriani et al., 2010). 'Muscat' varieties have been known and appreciated since ancient times (Scienza et al., 1989) and different theories exist regarding their origin as well as the name 'Muscat' (Dalmasso et al., 1964; Robinson et al., 2012). Nowadays, they are widely distributed around the world and

have a great importance. They are the basis of different appreciated wines and are also consumed as table grapes or as raisins.

In Spain, both varieties are authorized in several PDO (Protected Designation of Origin, a prestigious Spanish regional product classification) with a total extension of 1,892 hectares for 'Muscat à Petit Grains blanc' and 10,318 hectares for 'Muscat of Alexandria' (MAPAMA, 2017). 'Muscat à Petit Grains blanc' is mainly grown in Castilla la Mancha region whereas 'Muscat of Alexandria' has great importance at the Comunitat Valenciana (in 'Valencia' and 'Vinos de Alicante' PDO).

In la Marina Alta region, in the Alicante province, it was reported the presence of 'Moscatel' ('Moscatell') culture from 1696 (Cabrera, 1992). In this area, raisin production achieved high economic importance in the XIX century with exportations to several European countries as well as to Canada and USA (Chabás, 1972; Calvo, 2003). The phylloxera (*Daktulosphaira vitifoliae* Fitch) infestation damaged vineyards and this crop was replaced mainly by buildings. Nowadays, it is starting a recovery of the culture and traditions associated to wine and raisin production in this region. In this work, we report the results of an analysis of the genetic variability in 'Muscat of Alexandria' accessions, including clones from La Marina Alta that showed differential ampelographic traits.

MATERIALS AND METHODS

Plant Material

A total of ten clones of 'Muscat of Alexandria' were analyzed in this work. Clone E-110 was used as the certificate reference clone (provided by Viveros Cortés nursery). Clone 14E was from La Colección de Vides de El Encín; clones 8, 11, 16, 30, 59 and 61 were from La Marina Alta (Alicante); clone 13 was from a neglected vineyard from Guardamar (Alicante) and clone 15 from a selection program (SAT Selección Vitivinicola Valenciana nursery). An accession of 'Moscatel de Hamburgo' from la Casa de las Vides collection was used as outgroup.

SSRs (Simple Sequence Repeat or Microsatellites) profiles and AFLPs (Amplified Fragment Length Polymorphisms)

Fifteen SSR markers (VVS2, VVMD5, VVMD6, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG64, VrZAG79, VrZAG83, and VMC1b11) were used for amplification. DNeasy Plant Mini Kit (Qiagen) was employed for DNA extractions from young leaves. PCR reactions were performed using two sets of multiplex as described in Peiró et al. (2018) and products of PCR were visualized using gel electrophoresis carried out on an ABI 3100 platform (Applied Biosystems). The SSRs profile for each accession was compared with those in VIVC (Vitis International Catalogue), previous standardization using the clone E110 for identification.

The AFLP analysis was performed using as forward primer *Mse*I+N and as reverse primer *Eco*RI+N, after digestion with the proper restriction enzymes. Each 20-µL PCR reaction contained 0.5 µL of the pre-amplified DNA, 50 ng of labeled *Eco*RI+3, 30 ng of unlabeled *Mse*I+3 primer, 2 µL of 10× PCR buffer, 4 mM dNTPs, and 0.4 U of *Taq* DNA Polymerase. The cycling conditions of labeled-PCR were 1 cycle of 30 s at 94 °C, 30 s at 65 °C, and 1 min at 72 °C and a touch-down profile (11 cycles with -0.7 °C/cycle, annealing temperature) for the annealing step, followed by 23 cycles at 56 °C constant annealing temperature and, finally, an extension cycle of 30 min at 60 °C. Similarly to SSR, products were visualized using the ABI 3100 platform.

Unweighted Pair Group Method with Arithmetic mean (UPGMA) phenogram was made using genetic distances with PowerMaker software and plotted using TreeView software for SSRs and AFLP markers.

Genotyping by Sequencing (GBS) was performed using the endonuclease ApeKI (G/CWGC) and the sequencing platform Illumina HiSeq 2000 (Illumina Inc.). High quality reads were mapped to the reference genome developed by the French-Italian consortium (*V. vinifera* IGGP 12x).

RESULTS AND DISCUSSION

In the research project CGL2015-70843-R (UPV), we initiated different approaches in order to recover and conserve ancient varieties threatened by genetic erosion (Gisbert et al., 2018). Among the surveyed accessions, 'Muscat of Alexandria' as well as 'Grumer Moscatell', that have been located with different names, were found in old vineyards (Peiró et al., 2018; Jiménez et al., under revision). Old grapevines of 'Muscat of Alexandria' were also found in orchards of the Alicante province for table grape consume.

Clones of 'Muscat of Alexandria' were confirmed through SSRs, showing all of them similar SSR profile with the exception of the clone 11 which had a mutation in the SSR VrZAG64. As expected, AFLPs showed intravarietal variability. There existed differences between the clone of reference (E-110) and most of clones from La Marina Alta, which grouped also the clone 13 from a neglected vineyard from Guardamar. Among them, the clones 11 and 59 were more similar than the clones 16 and 30; the clone 13 was nearest of this second group whereas the clone 61 of the first one. The clone 15, from a nursery selection program, was the most different one, excluding the accession used as outgroup ('Moscatel de Hamburgo'). The clone 14, from la Colección de Variedades de Vid de El Encín Collection, was the most similar to the reference clone E-110 (Figure1). Differences between the groups including clones 11-59 and clones 16-30 are easily observed for growth aptitude in yields were all these clones were present. Clones from the first group had lower vigor and more compact clusters than the clones from the second one.

The GBS technology was also used to analyze the genetic diversity by generating from 2 to 4 million of reads per sample. In average, 85% of reads were mapped (mapping quality > Q20) to the reference genome developed by the French-Italian consortium (*V. vinifera* IGGP 12x). Around 40,000 SNPs were identified, with a coverage greater than 10X. Polymorphisms between and within 'Muscat of Alexandria' clones were obtained. The experimental validations of the identified SNPs will provide markers to accurately fingerprint these clones. The SNPs will also be suitable for association studies or to develop molecular markers useful in selection programs.

Conclusion

In this work we found genetic variability in 'Muscat of Alexandria' accessions from the Comunitat Valenciana, mainly in clones from La Marina Alta that showed different growth aptitude. All these variability can be of interest in order to select genotypes with differential traits. The experimental validations of the identified SNPs will provide markers to accurately fingerprint these clones. They will be also suitable for association studies or to develop molecular markers useful in selection programs.

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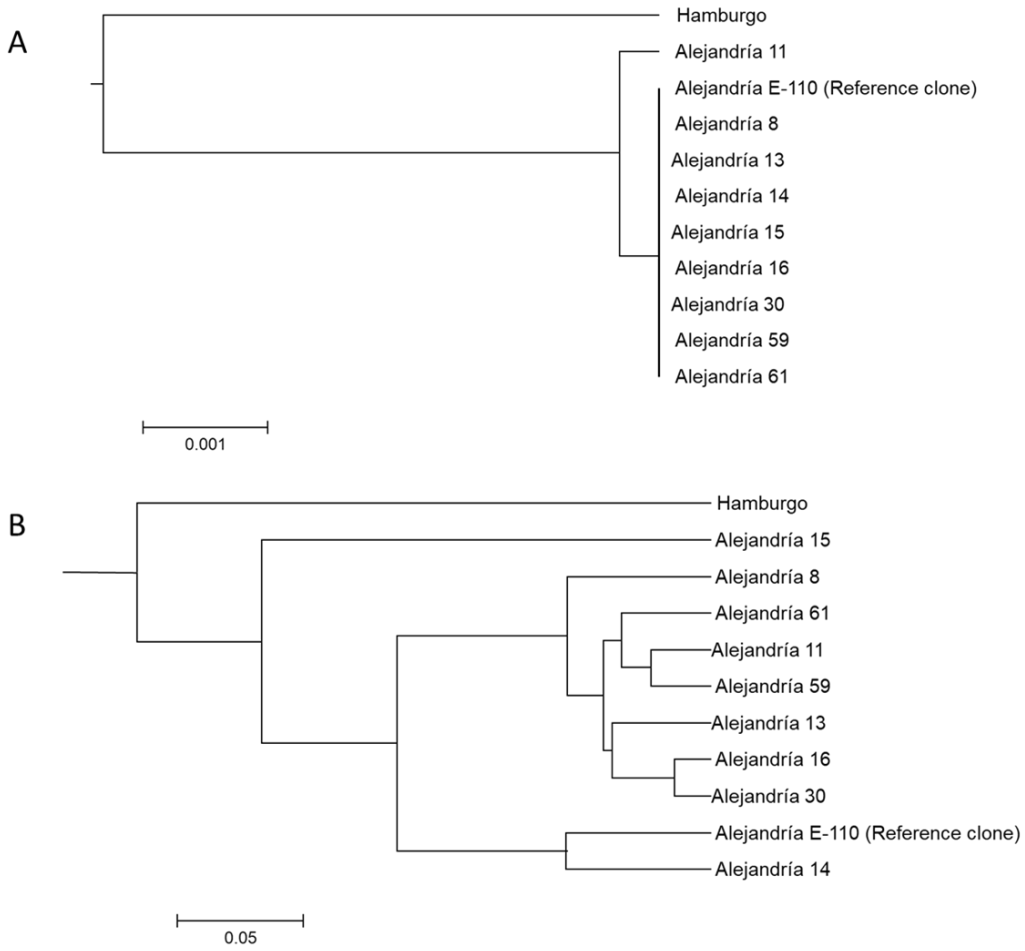

Figure 1. UPGMA (Unweighted Pair Group Method with Arithmetic mean) clustering results from the analysis of 10 'Muscat of Alexandria' accessions using SSRs (A) and AFLPs (B). 'Moscatel de Hamburgo' is used as outgroup.