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Pedigree reconstruction of wine and table grape crossbreeds created in Italy by Giovanni Dalmasso

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ABSTRACT

Starting in 1931, Giovanni Dalmasso carried out intense grapevine breeding activity, creating more than 100 wine and table grape crosses. He sought to contribute to the economic development of Italian viticulture, but also left considerable genetic material for further breeding programs.

Owing to the current strong interest in the genetic improvement of grapevines, the pedigrees of varieties released by breeders must be determined. The aim of this study was to genetically characterize and verify the disclosed pedigrees of Dalmasso's crosses (IDs). Nuclear microsatellite profiles (n-SSR) of 42 ID accessions and 22 genotypes declared as parents were obtained at 22 loci. By cross-validation of allele size, declared parentages were verified. When one or both disclosed parents were found to be incorrect due to inconsistent genetic data, putative parent(s) were sought via SSR profile comparison within the grapevine molecular database of CNR-Institute for Sustainable Plant Protection, and the probability estimated via IDENTITY v. 4.0 software.

Through microsatellite analysis, three duplicated genotypes were discovered and twenty ID parentages out of 39 were confirmed. In 13 IDs, one parent was incorrect, in 2 IDs, both parents were inconsistent with microsatellite profiles, and in 4 IDs the pedigree could not be verified since the pollen donor was not available. Apart from invalidated crosses likely due to pollen contamination, 5 accessions were mislabelled, either when both parents were invalidated or when two specimens of the same offspring were differently labelled. Verification of breeder's declared parentages revealed 43% of invalidated pedigrees within the investigated wine and table cross-breeds. The results provide additional insight into grapevine available diversity, and may aid the development of further breeding programs.

1. Introduction

Vegetative propagation of *Vitis* spp. varieties has been used for centuries, preserving the characters of the founder plants. Except perhaps in the early stages of domestication, or in cases of seed transportation by migrating populations, reproduction from seed has tended to be avoided, because of the uncertain traits exhibited by descendants. The grapevine is a highly heterozygous cross-pollinating species (This et al., 2006). However, cross-breeding is very advantageous for developing varieties with favourable traits, especially when pollination control and offspring's strict selection are applied. As far as is known, the first successes in grapevine genetic improvement by deliberate crossing date to the early nineteenth century, when Louis Bouschet obtained the two flesh-coloured varieties 'Petit Bouschet' and 'Gros Bouschet' (later used to develop other flesh-coloured wine grape varieties) (Alabouvette, 1936), and when other breeders, such as Foster in the UK and Vibert in France, released improved table grape cultivars.

After these pioneering successes, intense breeding activity, starting from the mid-nineteenth century, was chiefly directed at obtaining hybrids (mainly from North American species and *Vitis vinifera* L.) resistant to fungal diseases and phylloxera. Apart from breeding for resistance, during the last 150 years many Italian geneticists (Bruni, Cosmo, Dalmasso, Manzoni, Pirovano, Prosperi, Rigotti, Terzi, and others) as well as those from other countries (Branas, Gargiulo, Mathiasz, Müller, Olmo, Thomson, Truel, Vidal, to name a few) concentrated their cross-breeding programs on *Vitis vinifera* L., aiming to obtain new table grapes with interest-

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ing features, and new wine grape cultivars from which to produce appealing marketable wines.

Among the scientists mentioned, Giovanni Dalmasso started a cross-breeding program on wine and table grapes in 1931 at the Conegliano Viticulture Experimental Station (Veneto, North-Eastern Italy). The program was continued at the University of Turin (Piedmont, North-Western Italy). The program concerning table grapes aimed: a) to obtain improved genotypes from traditional varieties; b) to merge the grape quality characteristics from two or more genotypes (berry shape, color and flavour) with resilience and adaptability, c) to create new genotypes suited also to the cooler climate of Northern Italy. The wine grape program was chiefly concentrated on developing new crossbreeds combining positive technological features. According to Prof. Dalmasso's original notes, during twenty years of activity more than 100 wine and table grape crosses were selected, leaving a large body of grape diversity and considerable genetic material for further breeding.

Among these new cultivars, 14 wine grape varieties and 6 table grape genotypes are included in the Italian National Grapevine Variety Catalogue. Some of them, like the wine grape 'Albarossa' producing remarkable wines, currently enjoy the favour of growers and are greatly appreciated by the market. Others have never emerged from collections or experimental trials.

Among the most commonly used molecular markers for genetic analyses in grapevines, microsatellite alleles are inherited in Mendelian co-dominant segregation, confirming their suitability for genome mapping and to investigate hereditability and cultivar parentage (Thomas et al., 1994; Sefc et al., 2009). They can thus be used to confirm or invalidate breeder's information on the pedigree of a breed. Before the use of molecular markers, the putative parentage of new grape cultivars was retrieved from breeders' notes, which could be incomplete or inaccurate. Several studies have used microsatellite markers to clarify the parentage relationships between grape cultivars, either traditional or modern (Thomas et al., 1994; Cipriani et al., 2010; Lacombe et al., 2013). In grape-breeding programs, the female parent is certain, while the putative pollen donor might be mistaken because of contamination during pollination. This opens the possibility of a male parent not corresponding to the reported pedigree (Dettweiler et al., 2000; Ibàñez et al., 2009; Vargas et al., 2009; Lacombe et al., 2013). In some cases, due to mistaken varietal identity, the female parent may also be incorrect (Akkak et al., 2007). In connection with Dalmasso's crosses involving 'Nebbiolo' as parent, Torello Marinoni et al. (2009) discovered that, instead of the true 'Nebbiolo', Dalmasso accidentally used 'Nebbiolo di Dronero', a synonym for 'Chatus' that differs considerably from 'Nebbiolo'.

Because of the above errors, this study aimed to clarify the pedigrees of breeds obtained by Prof. Dalmasso that are still maintained in collections or commercially exploited. The secondary aim was to genetically characterize these materials, offering reference genetic fingerprints for the use of breeders, grape growers, and the wine industry. Due to the economic relevance of table grape breeding, there is worldwide interest in knowing varieties' correct pedigrees, because this information helps breeders to plan their cross-breeding programs.

As to the grape varieties discussed, many of them, especially the table grapes, are suitable for use in further breeding programs, because they exhibit improved traits in comparison to their parents (Carlomagno et al., 2014). At the same time, new varieties are more likely to be accepted and developed in the more dynamic table grape market. In recent years, much research activity is ongoing at the Department of Agriculture, Forest and Food Sciences (University of Turin, Italy), aimed to characterize the agronomic and qualitative behaviour of Dalmasso's table grape crosses in various environments, in the attempt to encourage their use at the local and national level.

Apart from their genetic value as a source of diversity, new wine grape cultivars could play a role in the more traditionalist wine market, through favourable features such as adaptability to climate change, or by offering an original, appealing flavour.

2. Materials and methods

2.1. Plant material

The SSR profiles of 42 Dalmasso interbreeds (IDs) and of 22 cultivars declared as parents were obtained. Samples from both IDs and declared parents were taken from field collections. In one case ('Pirovano 62') it was impossible to retrieve samples of the desired accession, because it was lacking in known collections.

The varieties (IDs) under investigation were described in terms of vine morphological features during the AGER Project, 2010–2104, "An Italian Vitis database with multidisciplinary approach, for exploitation and valorisation of the regional genotypes", employing the major OIV descriptors selected from the European *Vitis* Database (http://www.eu-vitis.de), and compared with published references.

Most of the plant material was taken from two collections maintained by the University of Turin, located at Chieri (Turin Province; planted in 1975) and at Alba (Cuneo Province; planted in 1984); 22 accessions were from the CNR-IPSP collection located at Grinzane Cavour (Cuneo Province; planted in 1992); two samples were kindly provided by CREA-VIT (Conegliano, Italy). After checking the uniformity of all the vines by morphology, for each accession one vine was chosen, from which to take a sample of young leaves, and the vine was labelled for further controls. Samples consisting of 3–4 young leaves were then stored at −80 °C until extraction; DNA extraction was done by the Thomas and Scott protocol (1993), slightly modified. Table 1 lists the 42 IDs analysed with their declared parents.

2.2. SSR analyses

Extracted DNA samples were amplified by Polymerase Chain Reaction (PCR) using primers labelled with four different fluorescent dyes (Fam, Ned, Pet, Hex). The PCR reactions were run in a BIO-RAD T100 thermal cycler, with the following thermal profile: one cycle at 95 °C for 3 min, followed by 28 cycles at 95 °C for 30 s each, 52 °C for 45 s, 72 °C for 90 s, and a final step of 30 min at 72 °C.

Amplification products were analysed on a 3130 Genetic Analyzer capillary sequencer (Applied Biosystems, Foster City, CA, USA). The internal GeneScan size standard 500LIZ (Applied Biosystems) was included in each run. Allele sizes in the output were determined using GeneMapper v. 4.0 software (Applied Biosystems), and alleles were designated by their size in base pairs (bp).

With regard to the markers used, at the first step, all IDs under study and their putative parents were analysed by the 9 SSR markers selected by the international scientific community for common use (This et al., 2004; Grapegen06: http://www1.montpellier.inra.fr/grapegen06/technical/index.html): VVS2 (Thomas and Scott, 1993), VvMD5, VvMD7 (Bowers et al., 1996), VvMD25, VvMD27, VvMD28, VvMD32 (Bowers et al., 1999), VrZag62 and VrZag79 (Sefc et al., 1999).

Declared parentages were verified by cross-validation of allele size. When one or both disclosed parents were found to be incorrect, due to inconsistent genetic data, the putative parent(s) were sought via SSR profile comparison within the grapevine molecular database of CNR-Institute for Sustainable Plant Protection (CNR-IPSP), which contains 850 unique genotypes of European cultivars (unpublished). At the second stage, all investigated IDs, their parents validated by the first 9 markers used, and the putative parents