Valorization of autochthonous Apulian grapevine cultivars for spumante production

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Abstract

Italian spumante has a long tradition, deeply rooted in the Piedmont region since the 19th century. A fundamental aspect of spumante production is berry acidity, since it influences both the quality and taste of spumante. The acidity of the berry results from the accumulation of H^+ ions in the vacuole of the cell, and this process involves complex biochemical changes not yet completely understood. Furthermore, acidification is strongly influenced by environment; in particular, higher temperature is associated with lower berry acidity. As part of a project aimed at evaluating and promoting Apulian cultivars for spumante production, six autochthonous grapevine cultivars ('Bianco d'Alessano', 'Bombino Nero', 'Maresco', 'Minutolo', 'Negramaro' and 'Uva di Troia') and two international cultivars ('Pinot Blanc' and 'Pinot Noir') were characterized both genetically, to constitute molecular fingerprints, and biochemically, to detect berry acidity values. The results obtained allowed us to identify the autochthonous cultivar 'Maresco' as the best candidate for comparison with the most commonly used cultivar for spumante production at the international scale, 'Pinot Blanc'.

Keywords: *Vitis vinifera*, Apulian varieties, spumante, acidification, transcriptomics

INTRODUCTION

Spumante is a wine with a long, deeply rooted tradition in Italy since the 19th century. Among the most used cultivars for spumante production in the world are the famous 'Pinot noir', 'Pinot blanc', 'Chardonnay' and flavored grapes like 'Moscato', 'Malvasia' and 'Traminer', all belonging to the species *Vitis vinifera*. The organoleptic characteristics of a spumante and its final taste are strongly influenced by several factors, such as berry acidity and flavor.

The processes that underlie grape berry development have not been completely deciphered, although recent omics analyses have contributed to their characterization and provided a better understanding of grape berry growth and ripening physiology (Deluc et al., 2007; Zenoni et al., 2010; Fasoli et al., 2012; Massonnet et al., 2017; Serrano et al., 2017; Balic et al., 2018). Grape berry development is characterized by two successive and distinct periods, during which the typology of solute accumulation changes dramatically. The first period, known as the herbaceous stage, is characterized by green and small berries that accumulate malic and tartaric acids, with a vacuolar pH around 2.5 (Rüffner, 1982). Herbaceous berries present a high rate of cell division, and carbohydrate respiration is the predominant metabolism, especially over malate synthesis (Koch and Alleweldt, 1978).

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During the second growth period, called ripening, berries start to change color and to accumulate soluble sugars such as fructose and glucose. In addition, fermentative pathways are induced (Sarni-Manchado et al., 1997). As a consequence, the vacuolar pH increases to 3.5, whereas the malate content is progressively reduced (Rüffner, 1982). The sugar-acid ratio is commonly used as a quality index in grape as well as in other fruit species. Specifically, berry acidity, which mostly depends on the concentration of H^+ ions in the vacuoles, plays a fundamental role in determining quality and taste of a spumante. The total acidity of grape berries comes from the complexity of stable (such as tartaric, malic, succinic, lactic and citric) and volatile (such as acetic) acids that will reveal the taste of wines, excluding carbon dioxide.

The aim of the present work was the evaluation of some Apulian grapevine cultivars suitable for production of spumante on the basis of their total acidity values. Six Apulian autochthonous genotypes, 'Bianco d'Alessano', 'Bombino Nero', 'Maresco', 'Minutolo', 'Negramaro' and 'Uva di Troia', were chosen and first genetically characterized through molecular markers. Two cultivars commonly used for spumante production, 'Pinot Blanc' and 'Pinot Noir', were also included in the analysis, as references. Molecular markers represent an ideal tool for accurate and exclusive characterization of cultivars; in particular, microsatellite or simple sequence repeat (SSR) markers are among the most suitable owing to numerous advantages such as their discrimination power, codominant nature, informative power, efficiency, wide distribution across genomes and ease of detection. For these reasons, nuclear SSRs have been largely applied to plant species for genetic variability studies (Stavrakaki and Biniari, 2017; Riaz et al., 2018), germplasm characterization (Regner et al., 2001; El Oualkadi et al., 2009; Maraš et al., 2014), varietal fingerprinting (Moravcova et al., 2006; Prins et al., 2009) and food traceability (Montemurro et al., 2015; Sabetta et al., 2017). The dual objective of this study was the constitution of a molecular fingerprint for each Apulian cultivar, based on the identification of unique and characterizing alleles, and their addition into the Apulian native cultivars database.

MATERIAL AND METHODS

Plant material

Young leaves and berries of each cultivar were collected from the experimental field of CRSFA Institute (Centro di Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia) located in Locorotondo (BA, southern Italy) and respectively stored at -20°C and -80°C prior to use. For acidification analysis, berries were collected at two time points: T_0, 35 daf (days after flowering), and T_1, 70 daf. For both time points, three biological replicates were taken. Sampling was carried out for two consecutive years. Material of the certified cultivars 'Pinot Blanc' and 'Pinot Noir' were used as references.

SSR analysis

Genomic DNA was extracted from young leaves according to the methods of Sabetta et al. (2011) and Pasqualone et al. (2016). DNA concentration and quality were checked both using a Nano-Drop™ 2000C Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and by 0.8% agarose gel electrophoresis. Eleven SSRs were chosen among the most informative, reproducible and polymorphic ones in the International Organization of Vine and Wine (OIV) database: VvS2 (Thomas et al., 1993), VvMD5 (Bowers et al., 1996), VvMD7, VvMD25, VvMD27, VvMD28, VvMD32 (Bowers et al., 1999), VvZAG21, VvZAG62, VvZAG64 and VvZAG79 (Sefc et al., 1999). Amplification reactions and capillary electrophoresis were performed as reported by di Rienzo et al. (2017). The electrophoretic outputs were analyzed using GeneMapper® 3.7 software.

Acidification analysis

The quantitative determination of total acidity was performed according to the Type I Method OIV-MA-AS313-01 reported in the Compendium of International Methods of Analysis (OIV, 2018).

RESULTS AND DISCUSSION

Genetic characterization

The first aim of this study was the molecular characterization, through the use of microsatellite markers (SSR), of some Apulian autochthonous grape cultivars. Five local genotypes were selected (‘Bianco d’Alessano’, ‘Bombino Nero’, ‘Maresco’, ‘Minutolo’, ‘Negramaro’ and ‘Uva di Troia’) together with two international cultivars (‘Pinot Blanc’ and ‘Pinot Noir’), and genomic DNA was extracted from young shoots and leaves of each cultivar.

The genetic characterization of the cultivars was performed by means of 11 SSR markers (VvS2, VvMD5, VvMD7, VvMD25, VvMD27, VvMD28, VvMD32, VvZAG21, VvZAG62, VvZAG64 and VvZAG79), chosen according to their reproducibility, quality of scoring, information content and discrimination capacity (http://www.oiv.int) (Schneider et al., 2014). Some examples of the electrophoregrams obtained are illustrated in Figure 1. Each autochthonous variety was identified by a unique and characterizing profile with a precise indication of allele molecular weights, thus making their unequivocal discrimination and correct denomination possible. When the molecular data were aligned to the “consensus list” for a comparison with international cultivars included in the European *Vitis* database (http://www.eu-vitis.de), a perfect match was found for three of the six local varieties, ‘Minutolo’, ‘Negroamaro’ and ‘Uva di Troia’, whose molecular profiles were identical to those reported in the database (data not shown). The molecular profiles of cultivars ‘Bianco d’Alessano’ and ‘Bombino Nero’ differed from those reported in the database for the same cultivars, for about 40% of alleles, thus indicating a certain level of genetic variability. Finally, cultivar ‘Maresco’ was not present in the database.

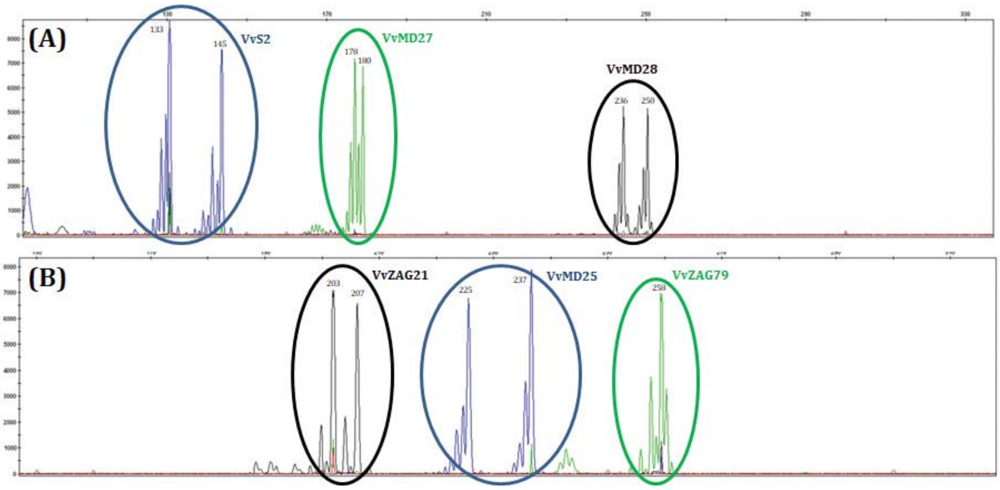

Figure 1. Examples of SSR electropherograms. Molecular profiles of cultivar ‘Bombino Nero’ at loci VvS2, VvMD27 and VvMD28 (A), and cultivar ‘Negramaro’ at loci VvZAG21, VvMD25 and ZAG79 (B). For each SSR, double (heterozygous) or single (homozygous) peaks are highlighted in the circles and allele dimensions are also reported.

The genetic similarity dendrogram (Figure 2) allowed us to cluster the analyzed cultivars in two main groups. The first group included ‘Maresco’, ‘Bombino Nero’ and ‘Negramaro’: although ‘Maresco’ is a white berry cultivar, while the other two have black berries, all these varieties are characterized by a high level of acidity and low sugar content (www.vitisdb.it). The second group was separated in two subclusters: one for ‘Uva di Troia’ and ‘Minutolo’, both characterized by high tannic and flavonoid contents (www.vitisdb.it), and one for the two Pinot genotypes, which showed identical molecular profiles. On the contrary, the cultivar ‘Bianco d’Alessano’ showed the highest degree of divergence, appearing as an out-group. It is a white berry genotype with low productivity, but high rusticity, adaptability to adverse environmental conditions and resistance to some biotic stress. Although its wine has an appreciable neutral color, the cultivar 'Bianco d'Alessano' is mostly used in blends for the production of some Apulian DOP wines.

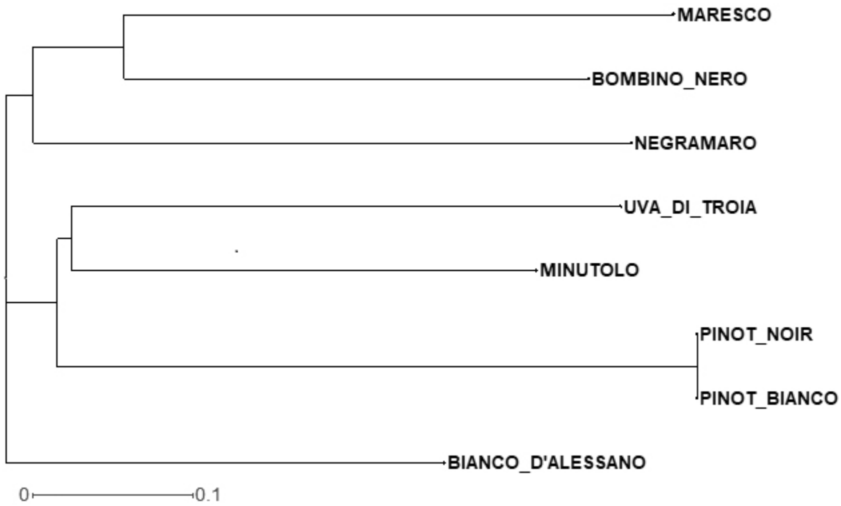

Figure 2. Genetic similarity dendrogram of six Apulian autochthonous grapevine cultivars plus two international cultivars, obtained by the analysis of 11 microsatellite markers.

Acidification

The total acidity of each wine obtained from berries of the analyzed cultivars was measured as the sum of all titratable acids, when a standard alkaline solution was added. Use of bromothymol blue as an indicator and comparison with an end-point color standard allowed adequate monitoring of the titration reaction. Acids in all samples were titrated and then expressed as tartaric acids. In Table 1, data related to the pH and tartaric acid content at two time points (T_0 and T_1) are reported.

Table 1. Quantification of total acidity (expressed as pH and tartaric acid content) in wines derived from six Apulian autochthonous genotypes and the two Pinot cultivars. Time points: T_0=35 daf; T_1=70 daf. Data are means of three biological replicates.

Cultivar	pH		Tartaric acid (g L^{-1})	
	T_0	T_1	T_0	T_1
Bianco d'Alessano	2.41	2.59	41.18	28.63
Bombino Nero	2.59	2.67	42.68	18.50
Maresco	2.63	2.74	50.53	30.91
Minutolo	2.78	2.74	41.12	27.65
Negramaro	2.57	2.52	44.10	30.96
Uva di Troia	2.63	2.76	44.18	34.59
Pinot blanc	2.53	2.81	24.68	19.89
Pinot noir	2.77	3.11	40.22	8.46

At the first time point, all the Apulian cultivars, except 'Minutolo', showed very low pH, comparable with that observed in 'Pinot noir'. Cultivars 'Minutolo' and 'Pinot noir' showed the highest pH. At T_1, pH of all cultivars was slightly increased, except that of 'Negramaro', which still remained rather low.

The tartaric acid content detected in all the cultivars at the first time point was generally high, as expected, and rather similar to that of 'Pinot noir'. Cultivar 'Maresco' could be clearly distinguished, since it showed the highest value (50.53 g L^{-1}). Surprisingly, the lowest value was observed for cultivar 'Pinot blanc' (24.68 g L^{-1}). At the second time point, as a consequence of metabolic changes and the increased sugar content of berries, the tartaric acid concentration decreased in all cultivars although by different amounts. In fact, the cultivars 'Maresco', 'Negramaro' and 'Uva di Troia' showed the highest tartaric acid content (30.91, 30.96 and 34.59 g L^{-1}, respectively).

Among the Apulian genotypes, the cultivars 'Maresco', 'Negramaro' and 'Uva di Troia' showed a tartaric acid content higher than that usually observed in 'Pinot blanc', one of the most commonly used cultivars for Italian spumante production. As it seems to be among the most promising candidates for the production of sparkling wine, the cultivar 'Maresco' has been chosen for further and future studies.

CONCLUSIONS

In view of the ever-increasing demand to promote Apulian autochthonous grapevine cultivars, a fingerprinting analysis was performed. This molecular analysis allowed clear distinction of all the cultivars studied, providing specific profiles for each of them.

Preliminary results regarding the total acidity of wine derived from each cultivar were useful for the identification of 'Maresco' as a promising candidate for spumante production. With the final aim to improve the quality of Apulian oenological products and to shed some light on the biosynthetic pathways involved in the complex acidification process of berries, a comparative transcriptomic analysis of the two cultivars 'Maresco' and 'Pinot blanc' will be carried out.

ACKNOWLEDGMENTS

This work was funded by the Apulian Region with the project "Aiuti a Sostegno dei Cluster Tecnologici Regionali" entitled "Innovazioni di processo e di prodotto nel comparto dei vini spumanti da vitigni autoctoni pugliesi" (project code: IProViSP-VJBKVF4).

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