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Research article

Metabolite profiling elucidates communalities and differences in the polyphenol biosynthetic pathways of red and white Muscat genotypes

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A B S T R A C T

The chemical composition of grape berries is varietal dependent and influenced by the environment and viticulture practices. In Muscat grapes, phenolic compounds play a significant role in the organoleptic property of the wine. In the present study, we investigated the chemical diversity of berries in a Muscat collection. Metabolite profiling was performed on 18 Moscato bianco clones and 43 different red and white grape varieties of Muscat using ultra-performance liquid chromatography–quadrupole time of flight–mass spectrometry (UPLC-QTOF-MS/MS) coupled with SNP genotyping. Principle component analysis and hierarchical clustering showed a separation of the genotypes into six main groups, three red and three white. Anthocyanins mainly explained the variance between the different groups. Additionally, within the white varieties mainly flavonols and flavanols contributed to the chemical diversity identified. A genotype-specific rootstock effect was identified when separately analyzing the skin of the clones, and it was attributed mainly to resveratrol, quercetin 3-*O*-galactoside, citrate and malate.

The metabolite profile of the varieties investigated reveals the chemical diversity existing among different groups of Muscat genotypes. The distribution pattern of metabolites among the groups dictates the abundance of precursors and intermediate metabolite classes, which contribute to the organoleptic properties of Muscat berries.

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1. Introduction

Natural variety in fruit metabolism is a fundamental aspect of crop breeding used to develop novel strategies for fruit quality enhancement. In tomato, exotic germplasm resources are being exploited for the identification of agriculturally valuable traits or for their potential use for metabolic engineering (Schauer et al., 2005). The screening of wild or natural varieties can be later used to generate mapping populations to study the genetic regulation of complex traits (Schauer et al., 2006). This approach was used to elucidate the genetic basis of fruit metabolism in several species (Schauer et al., 2006; Harel-Beja et al., 2010). In grape, the genomic resources available have increased significantly in recent years (Grimplet et al., 2009, 2011; Cipriani et al., 2008; Vezzulli et al., 2008; Denoeud et al., 2008; Cipriani et al., 2010; Laucou et al., 2011), and research efforts are dedicated to determine the genetic variation among grape varieties and its impact on fruit quality and metabolism (This et al., 2006). Yet, while extensive research has been conducted on the metabolism of developing berries of single varieties (Deluc et al., 2007), only a few works focused on the metabolic diversity between berries of different varieties (Mattivi et al., 2006; Cantos et al., 2002; Dimitrovska et al., 2011). Moreover, the distinguishing between the environmental and the

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genetic components and their interactions remains challenging (Vincent et al., 2007).

Muscat grapes are typically associated with a sweet floral flavor (Ribéreau-Gayon et al., 1975), which is, in large part, defined by its levels of geraniol, nerol and linalool (Gunata et al., 1985; Mateo and Jiménez, 2000). A general classification of Muscat-flavored varieties based on monoterpene concentration was proposed by Mateo and Jimenez (Mateo and Jiménez, 2000). Others characterized a collection of over 20 Muscat varieties for ampelographic-codified descriptors and for DNA polymorphism (Botta et al., 1999). Generally, the two characterization methods consistently categorized the varieties; nevertheless, in the white Muscat group, accessions with a different berry color showed the same DNA profile. Recently, the re-sequencing of 1-deoxy-D-xylulose 5-phosphate synthase (VvDXS), in a study on 148 grape varieties, which included Muscat-flavored, aromatic and neutral accessions, mutants and non-aromatic offsprings, revealed the key role of this gene on Muscat diversification (Emanuelli et al., 2010). The aforementioned studies add to the understanding of diversification of Muscat metabolism between varieties. Nevertheless a global analysis of the metabolic diversity of Muscat grapes is lacking.

A collection of 18 Moscato bianco clones and 43 Muscat varieties was established in Canelli, Piedmont (260 masl), grafted onto 'Kober 5 BB' and '420 A' rootstocks (Botta et al., 1999). The collection was intensively studied, and traits, including yield, percentage of bunches affected by gray rot and quality, i.e., the sugar content, total acidity, pH and terpene content, showed significant differences among the clones. Associations were eventually established between sugars and terpene compounds, while no significant relationship was noticed between total acidity, terpene content of the must and the percentage of Botrytis infection. Here, a UPLC-QTOF-MS-based metabolite profiling was used to characterize the varietal diversity in flavonoids and nonflavonoids of grape origins. The results are described on the background of the current knowledge of grape metabolism. Moreover, the metabolite analysis was coupled with SNP genotyping, using the 20K Infinium array from Illumina on a subset of the genotypes subjected to metabolic analysis.

2. Materials and ethods

2.1. Plant material and sampling

Samples were taken from a collection of Muscat grape varieties and clones located in Canelli, Italy. A set of varieties used both for wine and table consumption were included (Appendix A – Table A1). Sixty-one accessions were, examined, among which 18 were clones of Moscato bianco collected in Piedmont (Italy) and grafted either on Kober 5BB and 420A, 17 were synonyms or color mutants of Moscato bianco from different countries and the remaining 26 were different wine grape Muscat-flavored varieties and their synonyms. Berry maturity was monitored using a refractometer at weekly intervals during maturation. Samples were collected and measured for potential alcohol, titratable acidity, pH, berry weight and dimension over three years. All the samples were collected when the berries reached about 12% potential alcohol and were referred to by the date of designated maturity according to the winery (BOSCA) regulation. Each year berry weight was averaged from eight randomly selected berries of each genotype. Total soluble solids (TSS) in ^{o}Brix and potential alcohol (% Vol) were assayed yearly from the juice of the pooled crushed berries using a digital Maselli refractometer. Yearly titratable acidity measurements (g/L tartaric acid equivalents) were performed according to the standard procedures used in Guymon and Ough (Guymon and Ough, 1962). All the above berry quality measurements were repeated during three growth seasons (2010, 2011 and 2012). The list of the accessions included in the analyses, along with their country of origin, rootstock, berry color, parentage and their use, is presented in Appendix A (Table A1).

2.2. Metabolite extraction

Metabolic profiling was performed on berries from four independent replications (from different bunches) for each genotype during 2011 growing season. Each replicate included a pool of berry-skin tissue from five berries separated from the pulp. Samples were snap-freezed immediately with liquid nitrogen and kept at −80 °c until further analysis as described in Weckwerth et al. (2004). Skin tissue was dried in a lyophilizer, ground under pre-chilled holders and grinding beads using a RETCH-mill (Retsch Gmbh, 42787 Haan, Germany). A sample of 70 mg frozen tissue powder was transferred to 2 mL Eppendorf tube, and metabolites were extracted in a pre-chilled methanol/chloroform/water extraction solution (2.5/1/1 v/v/v). The mixture was then briefly vortexed and centrifuged for 2 min at 14,000 RPM (microcentrifuge 5417R), and the supernatant was decanted into the new tubes. The supernatant was mixed with 300 µl of chloroform and 300 µl of UPLC grade water and then centrifuged at 14,000 RPM for 2 min. The upper water/methanol phase was transferred to UPLC vials for LC-MS analysis.

2.3. UPLC–MS analysis

For LC-MS analysis, 4 µl of extracted sample was injected onto a UPLC-QTOF-MS system equipped with an ESI interface (Waters Q-TOF Xevo™: Waters MS Technologies, Manchester, UK) operating in negative and positive ion mode. Chromatographic separation was carried out on an Acquity UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 µm). The column and autosampler were maintained at 40 °C and 10 °C, respectively. During each sample running, the mobile phase comprised 95% water, 5% acetonitrile, 0.1% formic acid (phase A), and 0.1% formic acid in acetonitrile (phase B). The solvent gradient program was conditioned exactly as described previously (Hochberg et al., 2013). All analyses were acquired using leucine enkephalin for lock mass calibration to ensure accuracy and reproducibility, at a concentration of 0.4 ng L^{-1}, in 50/50 of ACN/H_2O with 0.1% v/v formic acid. The MS conditions were set essentially as described previously (Hochberg et al., 2013).

2.4. UPLC data processing

MassLynx software (Waters) version 4.1 was used as the system controlling the UPLC and for data acquisition as described previously (Hochberg et al., 2013). The raw data acquired were processed using MarkerLynx application manager (Waters) essentially as described previously (Hochberg et al., 2013). To verify metabolite identification, representative samples from each group were run using the same instruments and under the same operating conditions at the metabolomics facility of the Edmund Mach Foundation in San Michele all'Adige – Italy, where an in-house standard library described in details in Arapitsas et al. (2012), Degu et al. (2014) was used to validate metabolite annotation based on retention time order of commercial standards (Appendix A – Table A2), and metabolites were also identified based on a fragmentation pattern searched against the Chemspider metabolite database (http://www.chemspider.com/) and further confirmed (Appendix A – Table A3) with previous works (Sánchez-Rabaneda et al., 2004; Liang et al., 2008; Iijima et al., 2008; Hanhineva et al., 2008; Monagas et al., 2003, 2005; Moco et al., 2006). Each metabolite markers identified with the waters markerlynx software were then normalized to the total peak area in each run, on the internal

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