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Genotype/rootstocks effect on the expression of anthocyanins and flavans in grapes and wines of Greco Nero n. (*Vitis vinifera* L.)

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ABSTRACT

In the present study the effect of different rootstocks, namely 775 Paulsen, 779 Paulsen (*Vitis berlandieri* × *Vitis rupestris*), Ruggeri 225, 420 A and Kober 5BB (*Vitis berlandieri* × *Vitis riparia*) was evaluated on the anthocyanins and flavans profiles both in grapes and in relative wines obtained from Greco Nero n. vines grown in pots.

Rootstocks effects on secondary metabolites such as anthocyanins and tannins were quite evident both on grapes and on wines. The highest levels of anthocyanins was observed in berries of vines grafted onto 775 Paulsen and Kober 5BB. The corresponding wines presented greater color intensity, thus reflecting the anthocyanic content.

On the base of Principal Component Analysis, no distinction based on genetic background was evident on the base of anthocyanic profile both on grapes and wines. Conversely, grapes grafted on rootstocks Vitis *berlandieri* × *rupestris* differentiated from rootstocks Vitis *berlandieri* × *riparia* on the base of flavans variables, even this distinction was lost after winemaking.

The results of this paper suggested that a specific affinity between a certain genotype and a rootstock goes beyond the constitutive genetics of the rootstock itself and becomes peculiar of each scion/rootstock combination.

1. Introduction

Greco Nero n. (*Vitis vinifera* L.) is a wine grape variety principally cultivated in Calabria (Southern Italy), in the provinces of Catanzaro and Crotone, where it is known as Grecu Niuru or Maglioccone and recently gaining interest in Apulia region (Italy). It is a medium-late ripening cultivar (in the third decade of September) with medium-small size, short, conical and loose clusters, often with one or more secondary wings developed as the main one. The berries are medium, ellipsoidal and elongated with faintly pinkish berries. The skin is weaker thick, waxy, blue-black. The flesh is crisp with a pleasant taste (Schneider et al., 2008).

Different factors affect grape and wine performance in relation to vineyard microclimate, soil characteristics, training system, canopy management, soil nutrient availability, grapevine water status and shoot/rootstock interaction (Downey et al., 2006). Rootstocks normally derive from crosses between American Vitis species like *Vitis berlandieri* with *Vitis riparia* or *Vitis rupestris*. When grafted with Vitis *vinifera* L. genotypes, they confer resistant to biotic and abiotic stresses (Schmid et al., 1998; Reynold and Wardle, 2001; Corso and Bonghi, 2014).

Understanding the mechanisms underlying the phenotypic variability of a grape cultivar resulting from genotype/rootstock interactions means to deep information in terms of mobile signals, gene expression, and the genetic and epigenetic control of favourable changes in the vine above and below ground (Albacete et al., 2015). However, the molecular mechanisms associated with the ability of rootstock/scion combination to influence grape berry metabolism under drought stress are still poorly understood. (Berdeja et al., 2015). Many studies highlighted the role of the rootstock on leaf gas exchange with changes in net assimilation parameters, stomatal conductance, transpiration rate, hydraulic conductivity (Brown et al., 1985; Bavaresco and Lovisolo, 2000), regulation of K grapevine and in turn the juice pH (Kodur et al., 2013; Jogaiah et al., 2015; Tarricone et al., 2014). Further researches focused on the role of rootstocks on productive performances of vines and on relative phenolic compounds, anthocyanins and tannins (Koundouras et al., 2009; Ozden et al., 2010).

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Phenolic compounds in grapes vary according to the degree of ripeness reached (Ryan and Revilla, 2003), seasonal weather conditions (Cortell et al., 2007), soil type (Koundouras et al., 2006). Downey et al. (2006) reported that, beside light, temperature, altitude, soil type, water, nutritional status, microbial interactions, pathogenesis, wounding, defoliation, plant growth regulators, and various developmental processes, the most likely mechanism for decreasing phenolic content is excessive vine vigor. Therefore, an accurate vine canopy management is mandatory for a correct fruit exposure, nutrient availability, and water status of the plant. The possibility to transfer much phenolic compounds as possible from grapes to wines relies on the efficiency of the different protocols adopted in winemaking (Gambacorta et al., 2011). Vinci et al. (2008) summarized these aspects as follows. They indicated grape processing, like crushing, de-stemming, skin maceration, heating treatments, to be important steps influencing phenolic content in red wines. Similarly, the use of pectolytic enzymes and a right oxygen supply during ageing and storage revealed its efficiency in increasing phenolics in wines. Oxygen, in particular,has an essential role in improving large molecular weight phenolics (polymeric pigments), even a negative effect on antioxidant capacity of wines normally causes a reduction of low molecular weight phenolics, but on the other hand stabilizes the red wine color.

The main objective of this study was to evaluate the genotype/rootstocks interaction on the expression of anthocyanins and flavans both in grapes and wines of Greco Nero n. (*Vitis vinifera* L.).

2. Materials and methods

2.1. Experimental design, harvesting and grape sampling

One-year old cv. Greco Nero vines were planted in 2012 in 50 L plastic pots containing a mixture of organic soil and sand in equal proportions. All pots were placed in a 1.0 m by 1.5 m grid pattern exposed to full sunlight. During the experiments the grapevines were fertilized in autumn with a 100 g/vine of organo-mineral fertilizer NPK 10:5:14.5, manufactured by SCAM (Italy). Vines were grafted onto five different rootstocks: 775 Paulsen (775 P), 779 Paulsen (779 P), (*Vitis berlandieri* × *Vitis rupestris*) and 225 Ruggeri (225 Ru), 420 A, Kober 5 BB (*Vitis berlandieri* × *Vitis riparia*). From May to September 2015 the grapevines were watered by an automatic drip irrigation system (drip irrigation lines with one emitter per pot) which gave 4 mm of water per day during summer season. The pots were arranged in five rows of 10 vines each, with five vines per shoot/rootstock combination in each row. During dormant season vines were pruned (8 buds per vine) with a single cane and shoots were trained vertically on wires. At harvest, representative berry samples (1000 g) were drawn from each shoot/rootstock combination by pooling berries randomly picked from healthy and well developed clusters from the ten replicates. Half portion of the samples was used immediately for estimation of basic fruit composition such as total soluble solids (TSS), titratable acidity, pH, potassium etc. The other half of sample was divided in three subsamples in which berries were separated into skins, seeds and pulp for anthocyanins and flavans analyses.

2.2. Wine preparation

After harvesting, grape clusters for each of the five shoot/rootstock were pooled, cleaned and manually de-stemmed. The grape berries were passed through a small stainless steel presser to prepare must. Must of each thesis was divided into three replicates and each was inoculated with commercial yeast culture (*Saccharomyces cerevisiae*) with viable cell count, i.e. $1.06 \times 108\ mL^{-1}$. A microscale fermentation technique was used in 5 L stainless steel tanks at 20–22 °C. During fermentation process, fermenting material was mixed twice every day. The fermentation was completed by 10 days. The material like skin, seeds and yeast lees were separated from the finished wine. During the fermentation, temperature and must density were monitored every day. All vinifications and each treatments were performed in three replicates. After racking, wines were stored at room temperature for malo-lactic fermentation. Two month later the wines were racked, sulfur dioxide was added and were cold stabilized (−5 °C) for 1 month and then bottled. After further 2 months storage in bottles, the wines were analysed for alcohol content, total acidity, volatile acidity, reducing sugar, pH value, total SO_2 and total dry extract according to EEC Regulation 2676/90.

2.3. Chemicals and reference compounds

(+)-Catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, epigallocatechin, epigallocatechin gallate and malvidin chloride were purchased by Extrasynthese (France). Standards purities were all over 95%. All the solvents (methanol, acetonitril, ethyl acetate, diethyl ether, phosphoric acid) purchased by Carlo Erba (Milan, Italy) were HPLC grade. All the solutions were obtained with deionized water using Carlo Erba reagents.

2.4. Atomic absorption spectrometry analysis

Potassium, calcium and magnesium were determined by atomic absorption spectrophotometry. Analyses were performed by using a spectrophotometer AAS Perkin Elmer mod. Analyst 100. The procedures used for the preparation and calibrating refer to the official methods of analysis (Reg. CEE 2676/90).

2.5. HPLC-DAD analysis

2.5.1. Anthocyanins and organic acids

Anthocyanins and flavans were analysed on extract solutions obtained from seeds and skins of 20 berries per thesis, with three replicates, by putting seeds and skins tissues into 200 mL beakers containing 50 mL of a tartaric buffer solution at pH 3.20. The buffer solution was previously prepared by dissolving 5 g tartaric acid, 22 mL of 1N NAOH, 2 g sodium metabisulfite, and 125 mL 95% ethanol in distilled water up to a final volume of 1 L in a calibrated flask. The final pH was carefully controlled. Seeds and skins were kept in the buffer solution for 48 h (in the dark, at room temperature) and then filtered, brought at to volume 100 mL with the same buffer tartaric. The extract was stored in deep freezer at −20 °C for high performance liquid chromatography (HPLC) and spectrophotometric analysis (Di Stefano et al., 2008).

Anthocyanins from wines were isolated by passing 3 mL of wine through C-18 Sep-pak cartridge (da 300 mg) previously conditioned (by 2 mL di methanol e 2 mL H_2SO_4 0.01N), eluted with 3 mL methanol, evaporated to dryness (with Rotovapor) and redissolved in a mixture of 1 mL methanol: H_3PO_4 10^{-3} M (40:60) before injection into chromatographic system according to Squadrito's method (Squadrito et al., 2007). The samples were injected into a Thermo ODS RP-C18 Hypersil 100 × 2.1 (5 μm) column with a guard ODS Hypersil 20 × 2.1 mm (5 μm). Separation was carried out at 30 °C, the flow rate was 0.25 mL/min and the injection volume 10 μL. The detection was at 520 nm, using as solvent: A formic acid 10%; B formic 10% and methanol 50%. Linear gradients from 72% to 55% A in fifteen minutes; from 55% to 30% A in twenty minutes; from 30% to 10% A in ten minutes; from 10% to 1% A in five minutes; from 1% to 72% A in five minutes; equilibration time five minutes. The different analysed anthocyanins compounds were identified according to the retention time and the UV–vis spectral characteristics described in the literature (Gómez-Alonso et al., 2007; López