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Graft compatibility of *Vitis* spp.: the role of phenolic acids and flavanols

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ABSTRACT

The role of phenolic compounds in graft compatibility has been widely studied in various fruit species. However, little information is available for *Vitis* spp. This work aims at investigating the differences in phenolic acid and flavanol content in various scion/rootstock systems and understanding if they can be used as chemical markers of graft compatibility, using combinations of the Syrah cultivar (clones FR 470 and FR 383) with two rootstocks (110R and SO4). For this purpose, extracts of grafting tissues collected above graft union, at the graft union and below graft union from each combination at crucial stages of the grafting cycle (callusing, rooting and end of cycle) were prepared and analysed by HPLC. The results obtained revealed that the contents in gallic, ferulic and sinapic acids are closely related to the level of compatibility of the studied grafting combinations. The less compatible combinations (Syrah383/110R and Syrah383/SO4) exhibit higher content of gallic acid and lower contents of ferulic and sinapic acids than the more compatible ones (Syrah470/110R and Syrah470/SO4). In addition, these phenolic acids accumulated in the graft union and in the adjacent sections as a response to grafting. A significant difference in their levels was detected between the above and below graft union sections, especially at the rooting stage. Therefore, they can be seen as chemical markers to be used in an early detection of graft compatibility, preferably performed at the rooting stage and targeting the sections above and below the graft union.

1. Introduction

Grafting is widely used in horticulture, namely in the cultivation of grapevine and many fruit trees, assuring high yield, propagation and growth control. Grafting is also used because the rootstock can induce resistance to soil-borne pests, diseases and enhanced tolerance to abiotic stresses (Errea et al., 2000; Lee et al., 2010; Webster, 1995). Particularly in viticulture, it became widespread after a devastating infection of the soils by phylloxera. As American *Vitis* species were resistant to this pest, they started to be used as rootstock of *Vitis vinifera* L. cultivars (Legros, 1993). However, a lack of compatibility between the grafting partners may occur, causing economic losses that are difficult to overcome after the vineyard is already established. Several studies have been done in fruit species such as apple (Somelidou et al., 1994), pear quince (Musacchi et al., 2000), apricot (Errea et al., 2001; Usenik et al., 2006), loquat (Mng'omba et al., 2008), pear (Ciobotari et al., 2010), peach (Zarrouk et al., 2010) and cherry (Pina et al., 2012), but grapevine graft compatibility research has been poorly addressed (Cookson et al., 2013; Gokbayrak et al., 2007; Milien et al., 2012).

Graft compatibility can be defined as the establishment of a successful graft union resulting in a proper functioning composite, grafted plant (Goldschmidt, 2014). In practice, it has been found that different scion/rootstock combinations may have different levels of compatibility, i.e., different graft success rates. Successful grafting is a complex biochemical and structural process that begins with an initial wound response, followed by a callus formation, the creation of a continuous cambium and the establishment of a functional vascular system between the two grafting partners

Abbreviations: gal, gallic acid; ferul, ferulic acid; chlor, chlorogenic acid; caf, caffeic acid; sinap, sinapic acid; cat, (+)-catechin; epicat, (−)-epicatechin; TPI, total phenolic index.

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(Milien et al., 2012). Several processes need to occur at the graft interface such as cell recognition, initiation of the cell cycle, cell proliferation, cell differentiation and plasmodesmata development (Pina et al., 2009) in which some secondary metabolites, such as phenolic compounds, seem to be intensely involved (Bennett and Wallsgrove, 1994; Errea et al., 2000; Mng'omba et al., 2008; Moore, 1984, 1996; Usenik et al., 2006). It is known that phenolic compounds are part of the plant defence mechanisms (Errea et al., 1994), and their biosynthesis is triggered by stress situations such as wounding and infections (Bennett and Wallsgrove, 1994; Hart and Hillis, 1972; Haslam, 1979; Loehle, 1988). They play an important role in the graft-union formation during the callusing stage, where an intense production and subsequent accumulation of these secondary metabolites occurs. This accumulation in the wound tissues can cause marked effects on growth and metabolism of the shoots (Errea et al., 2000; Kahl, 1978). However, Moore (1996) stated that phenolic compounds can produce the same effects even if present in small concentrations. Some authors (Errea et al., 2001; Mng'omba et al., 2008; Usenik et al., 2006) pointed out that quantitative and qualitative differences in the phenolic patterns between the scion and the rootstock can imply dysfunctions at the graft union in different fruit trees. Unsuccessful grafting can occur, particularly in heterospecific grafts, and this lack of compatibility is associated with a pronounced accumulation of phenolic compounds above the graft union (Errea, 1998; Errea et al., 2001; Gebhardt and Feucht, 1982). For that reason, understanding the influence of phenolic compounds on graft establishment can be achieved through an analysis of the phenolic composition in each partner of a *Vitis* graft, allowing the development of a selection method for the more compatible combinations between a scion and a rootstock.

To the best of our knowledge, the first approach of the relationship between phenolic compounds and *Vitis* graft compatibility was recently performed by our team (Canas et al., 2015), through the development and validation of an analytical method for determination of phenolic compounds in *Vitis* grafting tissues. This method is able to distinguish graft combinations with different levels of compatibility based on the analysis of phenolic acids and flavanols at the callusing stage.

These promising results led us to continue with the study in order to get a deeper knowledge on the role of these phenolic compounds on the metabolism of the scion/rootstock system and to understand if they can be used as chemical markers of graft compatibility. Combinations between two clones of Syrah cultivar (FR 470 and FR 383) and two rootstocks—Richter 110 (110R, *V. berlandieri* × *V. rupestris*) and Selection Oppenheim 4 (SO4, *V. berlandieri* × *V. riparia*) were used, and quantification of the phenolic compounds was assessed at crucial stages of the grafting cycle, i.e., callusing, rooting and end of cycle.

2. Materials and methods

2.1. Plant material

The vegetative material used consisted of certified virus-free plants provided by the Plansel nursery, located at Montemor-o-Novo, Portugal (291 m above sea level, 38°39′N and 8°13′W) or acquired from a French grapevine nursery. All grafting procedures were executed in the Plansel nursery.

In April 2012, the plants were bench grafted using a grafting machine with the 'omega-cut' technique and dipped in paraffin at 75–80 °C to facilitate the sealing of the scion and rootstock. Then, the grafted plants were placed in forcing boxes, filled with peat and maintained in a dark room (3 weeks at approximately 30 °C and 80–90% relative humidity) in order to induce graft callusing.

Table 1
Samples description.

Combination Scion/Rootstock	Scion	Rootstock
Sy470/110R[a]	Syrah 470	110R
Sy383/110R[b]	Syrah 383	110R
Sy470/SO4[a]	Syrah 470	SO4
Sy383/SO4[b]	Syrah 383	SO4

[a] More compatible.
[b] Less compatible.

The grafted plants were transferred to the field where rooting stage took place (May 2012–July 2012), remaining there until the end of the grafting cycle (February 2013).

Four grafting combinations between scions of *Vitis vinifera* L. cultivar Syrah (clones ENTAV-INRA/FR 470 and 383) and rootstocks 110R (clone JBP/PT 2) and SO4 (clone ENTAV-INRA/FR 157) were studied (Table 1). The clones of cultivar Syrah were selected because they are prone to biotic and abiotic stress at different levels. According to Renault-Spilmont et al. (2005), the clone Syrah 383 has more problems in establishing a successful graft union than the clone Syrah 470. The rootstocks chosen have been described as having different levels of graft union success with Syrah: the rootstock 110R is no longer recommended to be used with Syrah while SO4 seems to have no special problem (ENTAV-INRA).

From each grafting combination, five groups of three plants (replications) were collected in the Plansel nursery at the end of the three stages: callusing stage, rooting stage and end of the cycle.

2.2. Sample preparation

Samples of the callusing stage were collected in May 2012, samples of the rooting stage were collected in July 2012, and samples of the end of cycle were collected in February 2013. The 60 plants of each stage (four combinations × five groups × three plants) were stored in a refrigerator at 4 °C, at INIAV-Dois Portos. On the day after the harvest, the plants of each combination were prepared according to the method described by Canas et al. (2015). Briefly, the plants were cut up 12 cm from the upper end, gathered (five groups × three plants) with proper identification, and stored in a refrigerator at −80 °C. Thereafter, paraffin was removed and three small sections with 1 cm length were obtained from each plant: 1 cm above graft union, corresponding to the scion; graft union; 1 cm below graft union, corresponding to the rootstock. They were cut longitudinally, and the bark and cortex were removed. The remaining tissues (xylem, phloem, and cambium) were rolled, immediately frozen in liquid nitrogen and ground in a mortar, in order to be analysed. The tissues of each section from the three plants of the same group were immediately mixed, weighed, wrapped in aluminium foil, frozen in liquid nitrogen and kept at −80 °C until extraction.

2.3. Chemicals

Gallic acid monohydrate, chlorogenic acid, caffeic acid, ferulic acid, sinapic acid, (−)-epicatechin, and scopoletin were purchased from Fluka (Buchs, Switzerland). (+)-Catechin was purchased from Sigma-Aldrich (Steinheim, Germany). All of them were used as standards (purity > 98%) without further purification. The standards were prepared fresh prior to use with methanol gradient grade (Merck, Darmstadt, Germany). The solvents used in the sample extraction and in the spectrophotometric analysis procedure were analytical grade. All solvents used in the chromatographic analysis were HPLC gradient grade purchased from Merck (Darmstadt, Germany). They were filtered through 0.45 μm membrane (Millipore, New Bedford, USA) and degasified in an ultrasonic bath.