Molecular advances in rootstock-scion interaction in grapevine

C. Marè, E. Mica and L. Cattivelli

Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CRA), Genomics Research Centre, Fiorenzuola d'Arda (PC), Italy.

Abstract

In grapevine, grafting is a worldwide used technique employed to confer resistance to pests and diseases, to improve abiotic stress tolerance and to control plant vigour and some qualitative traits. Despite the relevance of rootstock, knowledge of the molecular mechanisms implicated in the rootstock-scion interaction still has many gaps. Only, recently, some experiments have been performed to investigate the effects of grafting technique, different soils and rootstocks on scion at transcriptomic level. This work reviews the most relevant findings in this field. These recent advances open a new perspective for a molecular interpretation about the interaction between scion and rootstock as well soil substrates.

Keywords: *Vitis vinifera*, graft union, gene expression, soil, molecular trafficking

INTRODUCTION

Vitis vinifera development in cultivated systems is profoundly affected by selection of the appropriate rootstocks, chosen among different *Vitis* species, such as *V. berlandieri*, *V. riparia*, and *V. rupestris*. In addition to enhanced resistance to phylloxera, several traits of rootstocks from *Vitis* species have been selected by breeders to provide resistance to various pathogens, tolerance to abiotic stresses as drought, lime, salt, frost, high salinity and Fe^{2+} deficiency (Arrigo and Arnold, 2007; Corso and Bonghi, 2014). Many reports have indicated that grapevine rootstocks affect the growth of the *V. vinifera* scion in terms of vigour, yield, fruit development and quality and wine quality (Main et al., 2002; Gawel et al., 2000; Reynolds and Wardle, 2001; Ollat and Lafontaine, 2003; Tandonnet et al., 2010; Gregory et al., 2013). Grafting a cultivar onto a rootstock implies an interaction of two genetically different individuals, often belonging to different species. Since root traits (e.g., water and mineral uptake and transport) strongly influence leaves and fruit development both at physiological (e.g., stomatal conductance which in turn impact on the photosynthetic activity) and metabolic (accumulation of secondary metabolites) level, the same scion can express different phenotypic traits depending on the specific rootstock combination (Serra et al., 2014). Consequently, significant work has been carried out to select the most convenient rootstock/scion combinations to satisfy specific grape growing needs (Koundouras et al., 2006; Meggio et al., 2014).

The recent 'omics' technologies have allowed the genetic and functional characterization of different *Vitis* species as well as the molecular analysis of the rootstock-scion interactions (Deluc et al., 2009; Grimplet et al., 2009; Corso and Bonghi, 2014).

MOLECULAR EVIDENCES IN ROOTSTOCK-SCION INTERACTION IN GRAPEVINE

Despite the crucial role of the rootstock and soil in establishing a successful vineyard in terms of yield, plant longevity and wine quality, almost no molecular evidence linking the effect of the rootstock on scion to the gene expression have been reported in grape. Only recently, some microarray experiments (Marè et al., 2013; Cookson et al., 2013, 2014; Cookson and Ollat, 2013) have investigated gene modulation in response to grafting technique, different soils and rootstocks in *V. vinifera* opening a new perspective for the understanding of the factors controlling the grapevine features.

In the autografting system *Vitis vinifera* 'Cabernet Sauvignon N' grafted on itself (CS;

CS/CS) (Cookson et al., 2013), the transcriptome of grapevine rootstock and graft interface tissues were investigated. The samples were collected in the spring, 3 days and 28 days (when the callus is completed) after grafting of over-wintering stems. Generally, many genes were induced in the graft interface tissues compared with the rootstock, i.e., genes involved in cell wall synthesis, secondary metabolism, and signaling providing the evidence that the graft union formation induces transcriptional changes both in rootstock and graft interface tissue.

Afterwards, Cookson et al. (2014) have developed an experimental rootstock-scion system composed of two heterografting to verify whether grafting two different genotypes alters gene expression at the graft interface in comparison to the presumably wound-like transcriptome changes induced in autografts. In particular, *Vitis vinifera* 'Cabernet Sauvignon N' was heterografted on *Vitis riparia* 'Riparia Gloire de Montpellier' (RG;CS/RG), and on *Vitis rupestris* '1103 Paulsen' (1103P;CS/1103) in comparison with the autografting of 'Cabernet Sauvignon'. The samples were studied during the time course of the first month after grafting. Both heterograftings, compared to the autografting, trigger a modulation of several differentially expressed genes in the graft interface tissue up-regulating the genes related to oxidative stress response and PR proteins as well as many genes involved in plant stress responses. However, the transcriptome modulation triggered by the two different rootstocks RG and 1103P on the graft interface in CS was very similar. The findings of this work suggest that the cells at the graft interface can induce an immune-type response as effect due to the presence of a rootstock belonging to other species.

On the same experimental heterografting systems, the gene expression was investigated in the shoot apical meristems (Cookson and Ollat, 2013). Globally, the two different rootstocks, grafted on 'Cabernet Sauvignon', induced a huge modulation of gene expression in the shoot apex. The functional categories related to chromatin regulation, cell organization and hormone signaling were the most enriched in the up-regulated genes in the shoot apex of hetero-grafted plants. However, the variation of gene expression was similar in both heterograft combinations, highlighting that auto- vs. heterografting, rather than different rootstocks, was the major factor controlling the regulation of gene expression in the shoot apex.

The effects of rootstock-scion interaction on leaf transcriptome, one year after heterografting, was investigated detecting the variations in mRNA levels in a bi-factorial experiment using two rootstocks and three soil substrates (Marè et al., 2013).

The heterografting system, composed of budwood plants of *V. vinifera* 'Pinot Noir' (PN, clone ENTAV 115) grafted on *Vitis rupestris* '1103 Paulsen' (1103P; PN/1103P) or *Vitis riparia* × *Vitis rupestris* 'Millardet et de Grasset 101-14' (101-14 Mgt; PN/101-14 Mgt), were planted in pots filled with different soils: turf; sandy soil or a typical vineyard soil from Asti region (Italy), and grown in greenhouse. This bi-factorial experiment revealed that the rootstock effect on gene expression was relevant only in plants grown on sandy soil (256 genes differentially expressed between 1103P and 101-14). On the contrary, no transcriptional differences were perceived when the two rootstocks were compared on turf or on vineyard soil. When a single scion-rootstock combination was tested on different substrates, the results highlighted a large difference in gene expression between turf and other substrates, while almost no differences were detected between sandy and vineyard soils.

Sandy soil represents a non-optimal growing substrate, because it is devoid of nutrients and it has a low water holding capacity (Creasy and Creasy, 2009). Under these conditions plants can experience some degree of drought stress. On this substrate, a key feature of the response induced by 1103P compared to 101-14 rootstock was represented by a general up-regulation of the phenylpropanoid metabolism in the scions grafted on 1103P (Figure 1). The same pathway was down-regulated when plants grafted on 1103P and grown on turf were compared to the same plants grown on other substrates. Overall, 1103P rootstock grown on vineyard and sandy soils promoted the up-regulation of genes involved in pathways leading to the accumulation of several compounds with physiological activity as stress protecting agents, attractants or feeding deterrents.

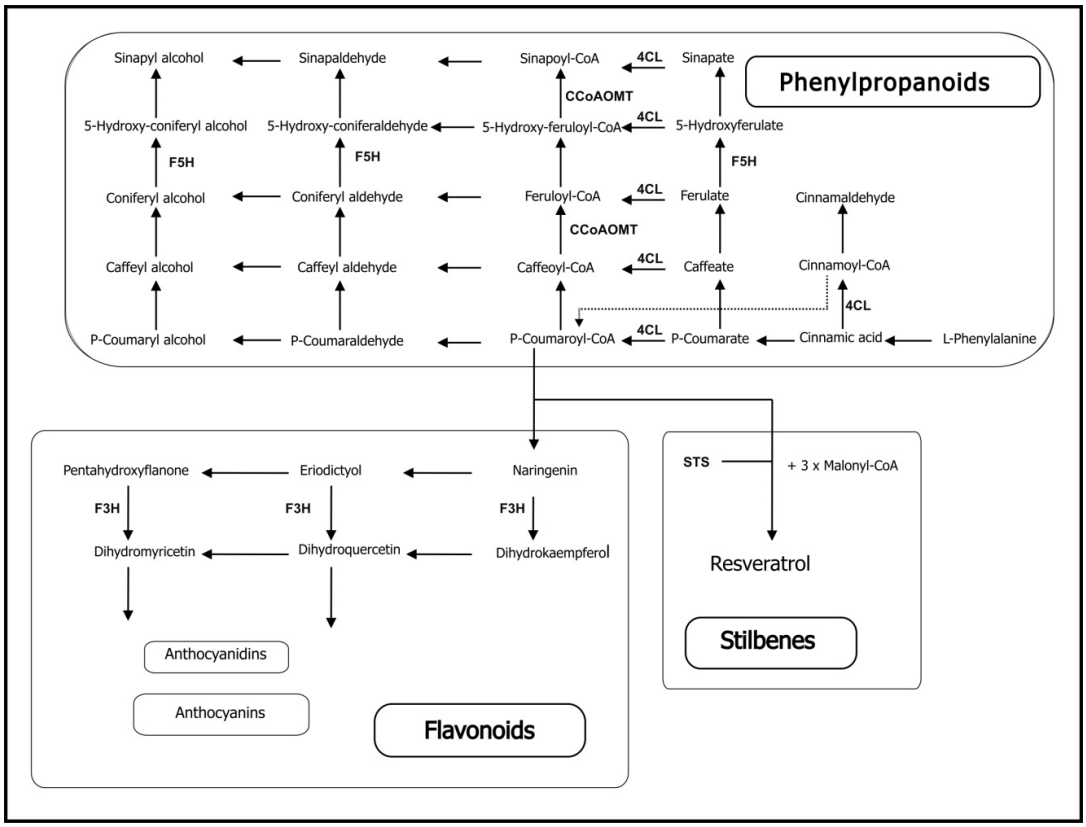

Figure 1. Schematic representation of phenylpropanoid pathway in *Vitis vinifera*. The acronyms identify the enzymes whose transcripts are up-regulated in leaves of 'Pinot Noir' grafted on 1103P rootstock (1103P;PN/1103P) in comparison with the leaves of 'Pinot Noir' grafted on 101-14 Mgt rootstock (101-14;PN/101-14 Mgt) grown on sandy soil. Abbreviations: 4CL, 4-Coumarate-CoA ligase; CCoAOMT, Caffeoyl-CoA-O-methyltransferase; F3H, Flavanone 3-hydroxylase; F5H, Ferulate 5-hydroxylase; STS, Stilbene Synthase (modified from Marè et al., 2013).

MOLECULAR TRAFFICKING ACROSS THE GRAFT UNION

The evidences described above demonstrate that rootstock has an impact on scion gene expression: this finding can be merely a consequence of a modification of the water and nutrient uptake due to the presence of the rootstock or could suggest that some signalling molecules can move across the graft union from rootstock to scion (and vice versa). The experiments of transgrafting, the use of a genetically engineered rootstock with a wild type-scion (or vice versa), have indeed demonstrated the transport of biological molecules from rootstock to scion across the graft union. Transgrafting has the potential to expand the traits provided by grafting since the benefits derived from transgenes can be harnessed. In the grapevine transgrafting system, composed of rootstock over-expressing the genes for polygalacturonases inhibiting proteins (pPGIP) thus tolerant to Pierce's Disease caused by *Xylella fastidiosa*, onto which a non-expressing scion is grafted, the pPGIP proteins, but not the pPGIP mRNA, were exported to the scion crossing the graft union via the xylem system (Agüero et al., 2005; Roper et al., 2007; Pérez-Donoso et al., 2010; Haroldsen et al., 2012).

Recent analytical technologies have revealed that some specific RNA molecules are also transported through phloem tissue as genetic information to promote coordinated organ growth and development. Evidences of highly regulated and selective processes involving long distance trafficking of mRNAs have been demonstrated (Kehr and Buhtz, 2007). Messenger RNAs encoding transcriptional regulators and cell fate/cycle-related,

hormone response, and metabolic genes have been identified in pumpkin and tomato sieve tube elements (Ruiz-Medrano et al., 1999; Kim et al., 2001; Haywood et al., 2005) as well castor bean (*Ricinus communis*, Doering-Saad et al., 2006), barley (*Hordeum vulgare*, Doering-Saad et al., 2002; Gaupels et al., 2008), and *Arabidopsis thaliana* (Deeken et al., 2008). These experiments have provided corroborative evidence for transport of mRNA via graft union providing strong evidences for the existence and function of a supracellular information signal migrating between the scion and the rootstock. Moreover, several microRNAs have been detected in the phloem sap of pumpkin (Yoo et al., 2004) and oilseed rape (*Brassica napus*; Buhtz et al., 2008, 2010; Pant et al., 2009) suggesting the potential signaling role of phloem in the long-range transport of gene expression regulators (Harada, 2010) (Table 1).

Table 1. Grafting systems used in the study of rootstock-scion interaction in grape and in horticultural species.

Rootstock	Scion	Tissues analysed	References
Cabernet Sauvignon N	Cabernet Sauvignon N	Rootstock	Cookson et al., 2013
V. vinifera	*V. vinifera*	Graft interface	
Riparia Gloire de Montpellier	Cabernet Sauvignon N	Graft interface	Cookson et al., 2013; 2014
V. riparia	*V. vinifera*	Shoot apical meristems	
1103 Paulsen	Cabernet Sauvignon N	Graft interface	Cookson et al., 2013; 2014
V. rupestris	*V. vinifera*	Shoot apical meristems	
1103 Paulsen	Pinot Noir	Leaves	Marè et al., 2013
V. rupestris	*V. vinifera*		
Millardet et de	Pinot Noir	Leaves	Marè et al., 2013
Grasset 101-14	*V. vinifera*		
V. riparia x *V. rupestris*			
Thompson Seedless –	Thompson Seedless	Xylem exudate –	Agüero et al., 2005
Chardonnay	Chardonnay	Leaves	Haroldsen et al., 2012
V. vinifera (genetically	*V. vinifera*		
engineered)			
Big Max – Pumpkin	Straight Eight – Cucumber	Scion phloem –	Ruiz-Medrano et al., 1999
Cucurbita maxima	*Cucumis sativus*	Apical tissues	Haywood et al., 2005
Mouse ears (Me)	*Xanthophyllic (Xa)*	Leaves	Kim et al., 2001
mutant – Tomato	mutant – Tomato		Haywood et al., 2005

CONCLUSIONS

While grafted scions and rootstocks are generally assumed to conserve their own genetic identity, it is becoming evident that certain transcription factors, mRNAs, regulatory microRNAs, small interfering RNAs (siRNAs), peptides, and proteins are mobile in the plant vascular system and thus, may cross the graft union. Latest microarray analyses highlighted a differential activation of genes in scion grafted on self and non-self rootstocks in grafted interface tissue and leaves. The autografting experiment suggests that the graft union formation modulates the transcriptome promoting cell wall synthesis, secondary metabolism, and signaling both in rootstock and graft interface tissue. Heterografting system analyses revealed that genes involved in defense and/or stress responses are specifically induced at graft interface when compared with autografted controls; nevertheless differential gene expression between different heterografting systems can be appreciated only in particular stress conditions. In the 'Pinot Noir' heterografting, the array analysis highlighted a differential activation of genes related to the phenylpropanoid pathway, to the carbohydrate and energetic pathways as well to the stress responsive mechanisms in grapevine leaves suggesting that soil and rootstock have an influence on scion transcriptome.

Improving molecular and biochemical knowledge, in addition to a deeper comprehension of the physiological aspects of the interaction between rootstock and grapevine cultivars will shed light into the biological mechanisms at the basis of the stress

resistance, thus allowing for the selection of genotypes that enable high quality also in unfavorable environments.

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