Journal of Plant Interactions

ISSN: 1742-9145 (Print) 1742-9153 (Online) Journal homepage: https://www.tandfonline.com/loi/tjpi20

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To cite this article: Caterina Marè , Alessio Aprile , Enrica Roncaglia , Emanuele Tocci , Lorenzo Giuseppe Corino , Luigi De Bellis & Luigi Cattivelli (2013) Rootstock and soil induce transcriptome modulation of phenylpropanoid pathway in grape leaves, Journal of Plant Interactions, 8:4, 334-349, DOI: 10.1080/17429145.2012.754958

To link to this article: https://doi.org/10.1080/17429145.2012.754958

Journal of Plant Interactions, 2013
Vol. 8, No. 4, 334–349, http://dx.doi.org/10.1080/17429145.2012.754958

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Rootstock and soil induce transcriptome modulation of phenylpropanoid pathway in grape leaves

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(*Received 3 October 2012; accepted 28 November 2012*)

Soil qualities and rootstocks are among the main factors that have been acknowledged to influence grape development as well as fruit and wine composition. Despite the role of soil and rootstock in establishing a successful vineyard in terms of grape quality, almost no molecular evidence linking soil and rootstock properties to the gene expression have been reported. The transcriptome variation in response to different soils and rootstocks was investigated through microarray technology. The cv. Pinot Noir was grown on different soils: sand, turf, and vineyard soil. The plants were grafted on the contrasting 101-14 and 1103 Paulsen rootstocks. The modulation of genes' expression in response to different soils and rootstocks was evaluated considering their potential impact on primary (carbohydrate) and secondary (phenylpropanoid) metabolisms. This study highlights a link among soil composition, rootstock, and gene expression. The results open a perspective for a molecular interpretation of the interaction between soil and grapevine.

Keywords: grape; phenylpropanoids; rootstock; soil; transcriptome

Introduction

Grape (*Vitis vinifera* L.) is an ancient cultivated species and one of the most economically important fruit crops worldwide. Soil qualities, rootstocks, topographical, agro-technical, and climatic factors have been acknowledged to influence grape development as well as fruit and wine quality (Rankine et al. 1971; Koundouras et al. 2006; Andrés-de-Prado et al. 2007). The soil provides the vine with nutrients and water availability, and therefore the soil composition of vineyards is considered a key factor for grapevines specificity. The influence of various soil properties, including texture, depth, chemical composition, water-holding capacity, water availability and its importance for the grapevine growth, and the characteristics and qualities of the wine have been widely studied (Turner & Creasy 2003; Andrés-de-Prado et al. 2007; Trought et al. 2008). For instance, it has been shown that soil water availability influences the grapevine hormonal equilibrium, while nitrogen, abiotic stresses, and water supply control the biosynthesis of flavonols and of other phenylpropanoid-derived products (Kao et al. 2002; Ubalde et al. 2010; DeLuc et al. 2011).

In light of the importance of the soil, the nature of the root system is a central issue for development, yield, and quality issues. In commercial vineyards the interaction soil/root system/canopy is complex because the relationships between *V. vinifera* scion and the soil are mediated by a rootstock that represents a different genotype with a genome that is, at least partially, different from the scion genome. Grapevine rootstocks were introduced to Europe after the phylloxera invasion, a pest which rapidly spread through vineyards, destroying large areas of most cultivars at the end of nineteenth century. Grafting European varieties on pathogen-resistant rootstocks is nowadays a normal practice and many varieties of rootstock have been developed by breeders. The more common American species used as rootstocks are crosses among *V. riparia* Michaux, *V. rupestris* Scheele, and *V. berlandieri* Planchon. Other species from Europe (*V. vinifera* L.) and Asia (*V. amurensis* Ruprecht) are also used. Several traits have been selected by breeders, such as resistance to phylloxera (*V. riparia*, *V. rupestris*, and *V. berlandieri*), nematodes, drought (hybrids *berlandieri-rupestris*), lime (*V. vinifera*), salt, and frost (*V. amurensis*) (Arrigo & Arnold 2007). As expected, numerous reports indicated that grapevine rootstocks affect the growth of the *V. vinifera* scion, yield, fruit quality, and wine quality (Ezzahouani & Williams 1995; Gawel et al. 2000; Reynolds & Wardle 2001; Main et al. 2002; Ollat et al. 2003). These effects are mainly indirect and are the result of a complex interaction between environmental factors and the physiology of both scion and rootstock cultivars

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This article was originally published with errors. This version has been corrected. Please see erratum (http://dx.doi.org/10.1080/17429145.2013.767645).

employed. Since the biochemical basis for the interaction is not yet clear, the selection of rootstock–scion combinations in fruit trees in general and in grapevines in particular is based on empirical testing. The recent introduction of the use of rootstock for vegetable crops is based on a similar strategy (Ruhl et al. 1988; Aloni et al. 2010; Harada 2010).

Despite the crucial role of the soil and rootstock in establishing a successful vineyard in terms of yield, plant longevity, and wine quality, almost no molecular evidence linking soil and rootstock properties to the gene expression of the grape cultivars has been reported.

The phenylpropanoid pathway is responsible for the synthesis of a large range of natural products in plants, including lignans, lignin, and flavonoids (Nugroho 2002). Flavonoids constitute a diverse family of phenolic molecules, whose compounds include six major subgroups found in most higher plants: chalcones, flavones, flavonols, flavandiols, anthocyanins, and condensed tannins [or proanthocyanidins (PAs)]. Stilbenes belong to a group of chemicals closely related to flavonoids and they are important secondary metabolites for plant defense. Besides, they play an important role in plant–environment interactions (Pourcel et al. 2007), flavonoids play key roles in signalling between plants and microbes, in the defense against predators and pathogens, in UV protection, in the flower and tissue pigmentation, and in the regulation of the auxin transport and in the scavenging reactive oxygen species (ROS) (Winkel-Shirley 2001; Nugroho 2002; Tattini et al. 2004; Pourcel et al. 2007). The defence-related flavonoids are constitutively synthesized but, additionally, their biosynthesis is often enhanced under the influence of several types of stress. They may also occur after pathogen infection, as the so-called phytoalexins (Treutter 2005). In grape, the most studied stilbene compound is resveratrol which is well known for its potential medicinal properties, particularly against cardiovascular diseases and cancer (Fornara et al. 2008). Many resveratrol derivatives, such as the viniferins, pterostilbene, and piceide, are also present in grape tissue and have been shown to be involved in plant defense mechanisms against biotic and abiotic stresses (Jeandet et al. 2002).

In this work the effect of different soils and rootstocks on the leaf transcriptome of the grapevine cv. Pinot Noir was analyzed to detect the variations in mRNA levels depending on soil and rootstock properties.

Materials and methods

Plant material and experimental design

The budwood plants (*V. vinifera* L., cv. Pinot Noir, clone 'ENTAV' 115) were grafted on February 2005 on different rootstocks: 101-14 Millardet et de Grasset (101-14) and 1103 Paulsen (1103P). 101-14 (*V. riparia* × *V. rupestris*) is a low-vigor rootstock characterized by low/moderate vigor, high *Phylloxera* resistance, low/moderate drought tolerance, very low salt tolerance, and early maturity. On the contrary, 1103P (*V. berlandieri* × *V. rupestris*) is a vigorous rootstock characterized by high vigor, good *Phylloxera* resistance, moderate/high drought tolerance, good alkalinity tolerance, moderate salt tolerance, and delayed ripening (Shaffer et al. 2004). Seventy grafts were produced for each rootstock. Afterwards, a selection of the best uniform grafts was made and 10 grafted plants for each of the 6 treatments (sandy soil with 1103P, turf with 1103P, vineyard soil with 1103P, sandy soil with 101-14, turf with 101-14, and vineyard soil with 101-14) were used to setup the experiment, and then three plants for each treatment showing a high level of visual uniformity were sampled for microarray analysis.

During May 2006 the young grafted grapes were planted in pots filled with different soils: turf, sandy soil, or vineyard soil from Asti (Italy), and grown in greenhouse. The soil chemical composition was analyzed, and the main characteristics are reported in Table 1. The pots' size was 30 cm diameter and 30 cm height, and the volume was 18.5 L. During the vegetative phase all pots were irrigated daily to restore

Table 1. Physical and chemical properties of the soils used in the experiment.

		Vineyard soil	Turf	Sandy soil
Particle size analysis (g/kg)	Sandy soil (2–0.05 mm)	255	207	626
	Silt (0.05–0.002 mm)	485	740	221
	Clay (<0.002 mm)	260	52	152
pH		8	5	8
Total limestone	(g/kg)	250	0	120
Organic matter (%)	(g/kg)	19	64	12
N	(g/kg)	0.6	1	0.6
C/N		18.5	37	11.5
P ass	(mg/kg)	2.5	902	4.7
K ass	(mg/kg)	172	8593	85
Mg ass	(mg/kg)	326	2336	103
Specific dry weight	(kg/L)	2.2	0.7	2.6
Specific wet (field capacity)	(kg/L)	2.5	1.2	2.8
Pots max weight (field capacity)	(kg)	40	19	45

the field capacity. The differences among scions, due to the contrasting vigor level of the two rootstocks, were not visually appreciated at the developmental stage as the plants were sampled, while the scions grown on turf soil showed a higher shoot elongation than scions grown on sandy or vineyard soils.

During the 2006 growing season the grapevines did not produce any fruits; therefore, the green fully expanded leaves were collected at different positions along the plant at the same time for all rootstock/soil combinations. The phenological stage of the plants at sampling corresponds to veraison stage which occurred in all plants during the following season in 2007. Three biological replicates were collected in 2006 for each condition and each replicate was a mix of several green leaves. Tissues were frozen in liquid nitrogen at harvest and stored at -80°C.

RNA isolation and array hybridization

Total RNA was extracted from leaves using a Tris–LiCl protocol (Tattersall et al. 2005) and was further purified using the Qiagen RNeasy plant mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. RNA quantity and integrity were confirmed by analysis on Agilent 2100 Bioanalyzer using RNA LabChip® assays (RNA 6000 nano Kit) according to the manufacturer's instructions.

RNA samples were processed following the Affymetrix GeneChip® Expression Analysis Technical Manual (Affymetrix, Inc., Santa Clara, CA, USA). Single-stranded, then double-stranded cDNAs were synthesized from the poly(A) mRNA isolated from 5 μg of total RNA for each sample using the Affymetrix one-cycle labeling kit and control reagents. The resulting *ds*-cDNA was column purified and then used as a template to generate biotin-tagged cRNA from an in vitro transcription (IVT) reaction using the Affymetrix GeneChip IVT labeling kit. The resulting biotin-tagged cRNA was fragmented and then hybridized at 45°C for 16 h (Affymetrix GeneChip Hybridization Oven 640) to probe sets present on an Affymetrix GeneChip® *V. vinifera* genome array. The arrays were washed and then stained (streptavidin–phycoerythrin) on an Affymetrix Fluidics Station 450 followed by scanning with a GeneChip Scanner 3000.

Data processing and analysis

Raw data from all hybridizations were normalized by Robust Multi-array Average (RMA) (Irizarry et al. 2003) using the R package Affymetrix library (Gautier et al. 2004). The same library was used to run the MAS 5.0 algorithm on raw data in order to produce a detection call for each probe set. On the basis of the detection calls (P = 'present,' M = 'marginal,' or A = 'absent') an initial filtering step was applied, because genes not expressed ('absent') represent experimental noise and can generate false positives. All probe sets that did not show a 'present' call in all reps of at least one sample were removed. R-squared linear correlation coefficients were computed on the RMA expression values for each set of biological triplicates.

RMA filtered data were imported to the software Genespring GX7.3 (Agilent Technologies, Santa Clara, CA, USA) for subsequent analyses. The rootstock effect on leaf transcriptome was determined carrying out three comparisons, one for each soil type (turf 101-14 vs 1103P, vineyard soil 101-14 vs 1103P, and sandy soil 101-14 vs 1103P). Similarly, the determination of the soil effect on leaf transcriptome was investigated with three comparisons (vineyard soil vs sandy soil, turf vs vineyard soil, turf vs sandy soil) for each rootstock. Differentially expressed probe sets were identified through a Welch *t*-test with Benjamini and Hochberg false discovery rate correction for multiple tests (Benjamini & Hochberg 1995). The differences in gene expression were considered to be significant when p-value <0.05 [see in Supplementary material 1: analysis of variance (ANOVA) test] and the induction or repression ratio was equal or higher than two-fold. Principal Component Analysis (PCA) was then employed to assess the role of rootstock and soil in the explanation of the variation in the data-set (Yeung & Ruzzo 2001).

Clusters of genes with distinctive expression patterns were searched with quality threshold (QT) cluster analysis. QT clustering algorithm groups genes into high-quality clusters based on two parameters: 'minimum cluster size' and 'minimum correlation.' The minimum cluster size was set to 20 and minimum correlation to 0.8.

To identify over-represented gene classes within selected clusters of genes compared to the entire array, the Arabidopsis best BLASTX annotations with *V. vinifera* probe sets was employed as input for the MIPS Arabidopsis thaliana database (MatDB, http://mips.helmholtz-muenchen.de/proj/funcatDB) (Ruepp et al. 2004). Only the annotations with a homology level cut-off equal or lower than 0.005 p-value were considered. Blast searches were done using PLEXdb (plant expression database http://www.plexdb.org) where a complete list of additional annotation MIPS functional categories for all probe sets on the *Vitis* genome array is available.

Validation of array data with real-time reverse transcription PCR (qRT-PCR)

Data validation was carried out on RNA extracted from the same biological replicates as for array hybridization. 4.5 μg of total RNA of each sample was reverse transcribed using oligo (dT)18 primer with M MLV reverse transcription reagents (Promega) according to the manufacturer's standard protocol. Subsequently, the cDNAs were quantified using a Qbit™ fluorometer (Invitrogen), diluted, and used for qPCR (quantitative polymerase chain reaction) amplifications with specific primers. qRT-PCR was performed with SYBR Green fluorescence detection

in a qPCR thermal cycler (ABI PRISM 7300, Applied Biosystems). Each reaction was prepared using 2 μL from a 2 ng/μL dilution of cDNA derived from the RT reaction, 12.5 μL of QuantiFast SYBR Green PCR Master Mix (QIAGEN), and 2.5 μL of forward and reverse primers 10 μM, in a total volume of 25 μL. Expression was determined for three analytical replicates. The cycling conditions were: 10 min at 95°C, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. Melting curve analysis was performed to evaluate the presence of nonspecific PCR products and primer dimers. The qPCR data were analyzed with ABI PRISM 7300 System SDS software. Analysis of relative gene expression was performed by fold change (FC) calculation using the following formula: $FC = 2^{-\Delta\Delta CT}$ where $\Delta\Delta C_T = (C_{T,\ \text{Target Gene}} - C_{T,\ \text{Reference Gene}})\ \text{Condition}_x\ (C_{T,\ \text{Target Gene}} - C_{T,\ \text{Reference Gene}})\ \text{Control Condition}$, where Condition_x is the value at any condition and Control Condition represents the 1 × expression of the target gene normalized to the reference gene. Specific qRT-PCR was carried for 16 probe sets corresponding to the following genes as described in the Results sections: *Caffeoyl-CoA-O-methyltransferase* (CCoAOMT) (1614643_at), *Flavanone 3-hydroxylase* (F3H) (1608379_at), *Stilbene Synthase* (STS) (1612804_at), *Glutathione S-transferases* (GSTs) (1614658_a_at and 1622015_at), *Glyceraldehyde-3-phosphate dehydrogenase* cytosolic (GAPDH) (1615814_at), *Phosphoenolpyruvate carboxylase* 1 (PEPC1) (1611103_at), *Phenylalanine ammonia-lyase* (PAL) (1619642_at), *Cinnamoyl-CoA reductase* (CCR) (1614423_at), *Chalcone synthase* (CHS) (1617019_at), *Chalcone-flavonone isomerase* (CHI) (1615912_at), *Flavonol synthase* (FLS) (1608791_at), *MYB-transcription factor protoanthocyanidins* (VvMYBPA1) (1616094_at), *Vacuolar invertase 2* (GIN2) (1612836_at), *Heat shock protein* (HSP18.2) (1612385_at), and *Galactinol synthase* (GolS) (1609808_at). qRT-PCR data were compared to the corresponding microarray expression values by mean of Pearson's product–moment correlation coefficients. Data were calculated from the calibration curve and normalized using the expression curve of the ubiquitin transcript (TC54117) corresponding to 1616334_a_at probe set, selected as reference gene in grape (Faccioli et al. 2010). The expression of the ubiquitin gene assessed with array data was equal and stable across all treatments. The ANOVA test confirmed that no statistical significant differences in ubiquitin expression existed among treatments (*F*-value 1.739, Pr $>F$ 0.2). The primer sequences, the results of the amplification, and the comparison with microarray data are displayed in Supplementary material 2: Gene expression validation by qRT-PCR.

Results

Microarray quality analysis

Global gene expression analysis was performed using the Affymetrix Vitis Genome Array. GeneChip® hybridization quality was verified using the standard Affymetrix controls. All hybridizations showed the expected checkerboard pictures. The collected data were normalized using RMA algorithm. The average background was 44.04, well within the recommended levels. The percentage of 'present' calls ranged between 64% and 71.8% among the 16k probe sets of the array. To value the quality of biological replicates, R-squared was calculated between the replicates of the same sample and the values ranged between 0.95 and 0.98 with an average value of 0.975.

Soil composition and rootstock type affected gene expression in the leaves of the scion

Three main sources of variation explaining 74.39% of total variance were identified by PCA. The two main components explained 30.85% and 24.4% of variance. The first component summarized the differences due to soil composition, while the second one reflected the differences due to rootstocks (Supplementary material 3: PCA).

Overall, 771 probe sets, each of them representing a putative gene, were differentially modulated at least in one experimental comparison (Table 2) (the list of all regulated genes is presented in Supplementary

Table 2. List of the differentially expressed probe sets in the different comparisons.

	Probe sets >2 × up-regulated	Probe sets >2 × down-regulated	Total regulated probe sets
Rootstock comparisons			
Turf soil: 101-14 vs 1103P	0	0	0
Vineyard soil: 101-14 vs 1103P	0	2	2
Sandy soil: 101-14 vs 1103P	108	148	256
Soil comparisons			
101-14: vineyard soil vs sandy soil	0	14	14
101-14: turf vs vineyard soil	97	137	234
101-14: turf vs sandy soil	3	25	28
1103P: vineyard soil vs sandy soil	0	0	0
1103P: turf vs vineyard soil	36	220	256
1103P: turf vs sandy soil	72	193	265

Note: Soil types: vineyard soil, turf, and sandy soil. Rootstocks: 101-14 and 1103P.

material 4: Differentially expressed genes in soil and rootstock comparisons).

No gene was modulated in all three rootstock comparisons; therefore, there was no evidence of a general effect of the rootstock across soil types. However, the effect of rootstock on gene expression was significant in plants grown on sandy soil. On the contrary, comparisons of the rootstocks in turf and vineyard soils did not highlight almost any probe sets which exceed the statistically significant threshold. In sandy soil, the comparison yielded 108 probe sets more expressed in Pinot Noir grafted on 101-14 rootstock and 148 probe sets more expressed in Pinot Noir grafted on 1103P rootstock (Table 2).

Concerning the soil comparisons, turf vs vineyard soil yielded a similar number of differentially expressed genes in 101-14 (234 probe sets) and 1103P (256 probe sets), while the comparisons turf vs sandy soil yielded much more differentially expressed genes in 1103P (265 probe sets) than in 101-14 (28 probe sets). The third comparison (vineyard soil vs sandy soil) yielded very few differentially expressed genes in 101-14 (14 probe sets) and none in 1103P (Table 2).

QT-cluster analysis was performed with all differentially expressed genes (771) to identify groups of genes that show a similar expression profile. The analysis yielded 12 clusters plus 318 unclassified probe sets. The cluster analysis has allowed the identification of some expression profiles corresponding to the two experimental factors (soil and rootstock): clusters 1, 2, 5, 9, and 11 contain a total amount of 227 genes whose expressions were mainly driven by soil (Supplementary material 5: Clusters_A), while clusters 3, 4, and 8 represent 114 genes whose expressions were mainly affected by rootstock (Supplementary material 5: Clusters_B), and the remaining clusters show an intermediate expression behavior (Supplementary material 5: Clusters_C).

The probe set analysis carried out by MIPS functional categories tool for each cluster identified several overrepresented categories (0.005 *p*-value cut-off). The following overrepresented functional categories were identified: 01 METABOLISM (subcategories 01.02 secondary metabolism, 01.20.35 metabolism of secondary products derived from L-phenylalanine and L-tyrosine, and 01.05 C-compound and carbohydrate metabolism), 02 ENERGY (02.01 glycolysis/gluconeogenesis and 02.10 TCA pathway), 34 INTERACTION WITH THE ENVIRONMENT (34.11 cellular sensing and response to external stimulus, 34.11.01 photoperception and response, 34.11.10 response to biotic stimulus), and 32 CELL RESCUE, DEFENSE, AND VIRULENCE (32.01 stress response, 32.01.05 heat shock response, and 32.01.01 oxidative stress response).

In the next paragraphs, attention will be paid to several metabolic pathways characterized by genes modulation in response to the variations in soil or rootstock traits.

Key genes of the phenylpropanoid pathway were up-regulated in scions grafted on rootstock 1103P vs 101-14 when grown on sandy soil

The leaves of Pinot Noir scions grafted on the vigorous rootstock 1103P and grown on sandy soil up-regulated the expression of several key genes involved in the phenylpropanoid metabolism (Supplementary material 6: Phenylpropanoid pathway-related ps) in comparison with the plants grown on the same type of soil and grafted on the low-vigor rootstock 101-14 (Table 3 and Figure 1).

All phenylpropanoids are derived from cinnamic acid, which is formed by phenylalanine by means of PAL. From cinnamate several simple phenylpropanoids, such as *p*-coumarate, caffeate, ferulate, sinapate, and simple coumarins, are produced so that, in turn, they lead to the biosynthesis of all phenylpropanoid compounds. Among the probe sets up-regulated in the scions grafted on 1103P, there were some corresponding to *4-Coumarate-CoA ligase* (4CL), *Caffeoyl-CoA-O-methyltransferase* (CCoAOMT), and *Ferulate 5-hydroxylase* (F5H) (Figure 1).

The stilbene synthases, the key enzymes of phytoalexin biosynthesis, catalyze the resveratrol biosynthesis, a naturally occurring phytoalexin and antioxidant molecule (Figure 1). The probe sets for *stilbene synthases* (STS1, STS2) transcripts were up-regulated in 1103P grafted plants in comparison with the plants grafted on rootstock 101-14.

Flavonoids are synthesized from phenylpropanoid derivatives. The naringenin is the starting compound for flavonoid biosynthesis. The probe sets corresponding to *Flavanone 3-hydroxylase* (F3H) and *Flavonol 3-O-glucosyltransferase* (F3-O-GT) (UDP-glucose: flavonoid 3-O-glucosyltransferase) were up-regulated in 1103P grafted plants. F3H is involved in the activation of anthocyanin biosynthesis catalyzing the transformation of naringenin into dihydrokaempferol, a driver molecule to flavonoid, flavone, and flavonol, and anthocynidin biosynthesis. Furthermore, F3-O-GT, adding a molecule of glucose to the 3-O position of a variety of flavonols as quercetin, and kaempferol, is involved in the flavonol pathway (Figure 1) (Offen et al. 2006).

Besides probe set-encoding enzymes, the transcriptomic analysis also identified a probe set corresponding to *VlMYB transcriptor factors B1*/*B2* (Deluc et al. 2009) more expressed in plants grafted on 1103P compared to the same plants grafted on 101-14 and grown on sandy soil. The *mybB transcription factors,* together with the *myb*-related genes, are involved in the regulation of anthocyanin biosynthesis in grape via expression of *UDP-glucose:flavonoid 3-O-glucosyltransferase* (*UFGT*) gene critical for anthocyanin formation in all red cultivars, but not in the white ones (Kobayashi et al. 2002) (Table 3).

The results indicate that under specific soil conditions (sandy soil) the rootstock affects gene expression of the scion leaves altering the expression profile

Table 3. List of the genes involved in the phenylpropanoid biosynthesis up-regulated in leaves of Pinot Noir grafted on 1103P compared to the same plants grafted on 101-14 when grown on sandy soils.

Gene annotation	Probe sets	FC sandy soil 1103P/101-14	BLASTN DFCI gene index release 7	E.C. number	UniProt
4-Coumarate-CoA Ligase (4CL)	1609307_at	2.09	TC119736	6.2.1.12	O24145
Caffeoyl-CoA-O-methyltransferase (CCoAOMT)	1614643_at	2.31	TC106122	2.1.1.104	Q43237
Flavanone 3-hydroxylase (F3H)	1608379_at	5.25	TC112113	1.14.11.9	Q8H8H7
	1608761_at	6.19	TC112113		
Ferulate-5-hydroxylase (F5H)	1620245_at	2.07	/		Q8W228
	1614502_at	3.11	TC107180	1.14.13.-	Q9SBP8
	1614045_at	3.71	TC127813		Q9SWR1
Flavonol 3-O-glucosyltransferase (F3-O-GT)	1615401_at	2.04	TC116915	2.4.1.91	Q40285
Stilbene Synthase 1-2 (STS1-2)	1608009_s_at	4.02	TC109969		P51070
	1609696_x_at	6.52	TC118801		P28343
	1610850_at	5.73	TC118801		P28343
	1611190_s_at	5.92	TC118801	2.3.1.95	Q94G58
	1612804_at	4.63	TC109969		Q9SPW2
	1620964_s_at	5.95	TC118801		P28343
	1622638_x_at	4.99	TC109969		Q9SPW2
VlMYB transcription factor B1-B2 (VlMYBB1-B2)	1620319_s_at	3.12	TC118242	/	Q8L5N7
Glutathione S-transferase (GST)	1622015_at	6.03	TC110049	2.5.1.18	Q4LAW7
	1610989_at	2.59	TC118101		Q03662

Note: The listed enzymes are displayed in Figure 1. The fold change (FC) value is calculated as sandy soil 1103P vs 101-14.

of genes involved in the phenylpropanoid biosynthesis with a specific focus on stilbenoid compounds.

Turf soil led to a down-regulation of genes of the carbohydrate metabolism

Three soil comparisons showed a large effect in terms of gene expression: turf vs vineyard soil on both rootstocks 101-14 and 1103P and turf vs sandy soil on 1103P (Table 2). The analysis of the genes modulated in these three comparisons highlighted the presence of 45 down-regulated probe sets when the plants are grown on turf compared to other substrates (vice versa the genes were up-regulated in plants grown on vineyard and sandy soils vs turf). These probe sets are enriched in genes annotated as components of the carbohydrate metabolism (Supplementary material 7: Carbohydrate metabolism glycolysis/gluconeogenesis TCA pathway-related ps). Six probe sets were putatively involved in the glycolisis/gluconeogenesis pathway: *Pyrophosphate-dependent 6-phosphofructose-1-kinase* (PPi-PFK), Glyceraldehyde-3-phosphate dehydrogenase cytosolic (GAPDH), *Phosphoglycerate kinase* cytosolic (PGK), Phosphoenolpyruvate carboxylase 1 (PEPC1), *Phosphoenolpyruvate carboxylase kinase 2* (PEPCK2), and *Sucrose-phosphate synthase 1* (SPS1) (Table 4, Figure 2). PGK, GAPDH, and PPi-PFK control reversible reactions, whereas PEPC1 and PEPCK2 are one-way antagonist enzymes that catalyze the transformation of phosphoenolpyruvate (PEP) to oxaloacetate (OAA) and vice versa. OAA, an intermediate of TCA cycle, is reduced to malate by cytosolic malate dehydrogenase (MDH), and is a cytosolic intermediate of glyoxylate cycle directed toward chloroplast, mitochondria, and vacuole (Sweetman et al. 2009). Moreover, the one-way SPS catalyzes the reaction from UDP-glucose to sucrose-6P, the penultimate step of sucrose synthesis, as a result of photosynthesis in the leaves (Davies & Robinson 1996).

The phenylpropanoid pathway was down-regulated in scions grafted on 1103P and grown on turf vs other substrates

Two (1103P in vineyard soil vs turf and 1103P in sandy soil vs turf) of the three comparisons described above share additional 51 genes commonly down-regulated in plants grown in turf (vice versa these genes were up-regulated in plants grown on vineyard and sandy soils vs turf), and, among them, some probe sets were annotated as components of the phenylpropanoid pathways (Supplementary material 6: Phenylpropanoid pathway-related ps). *PAL*, *CCoAOMT*, *CCR*, *CHS*, *F3-O-GT*, and GSTs were all down-regulated in 1103P grafted scions grown in turf vs other substrates (Figure 1, Tables 3 and 5).

PAL codes for enzyme responsible for the synthesis of *trans*-cinnamic acid, the primary intermediary in the biosynthesis of phenolics. Usually, as a consequence of various biotic and abiotic stresses, an increase in PAL activity and accumulation of many phenolic compounds were observed (Wen et al. 2008).

Two genes of lignin biosynthesis were regulated in response to soil type in 1103P grafted scions. *CCoAOMT* drives the feruloyl-CoA and sinapoyl-CoA biosynthesis and *CCR* leads to lignin biosynthesis transforming the sinapoyl-CoA to sinapaldehyde,

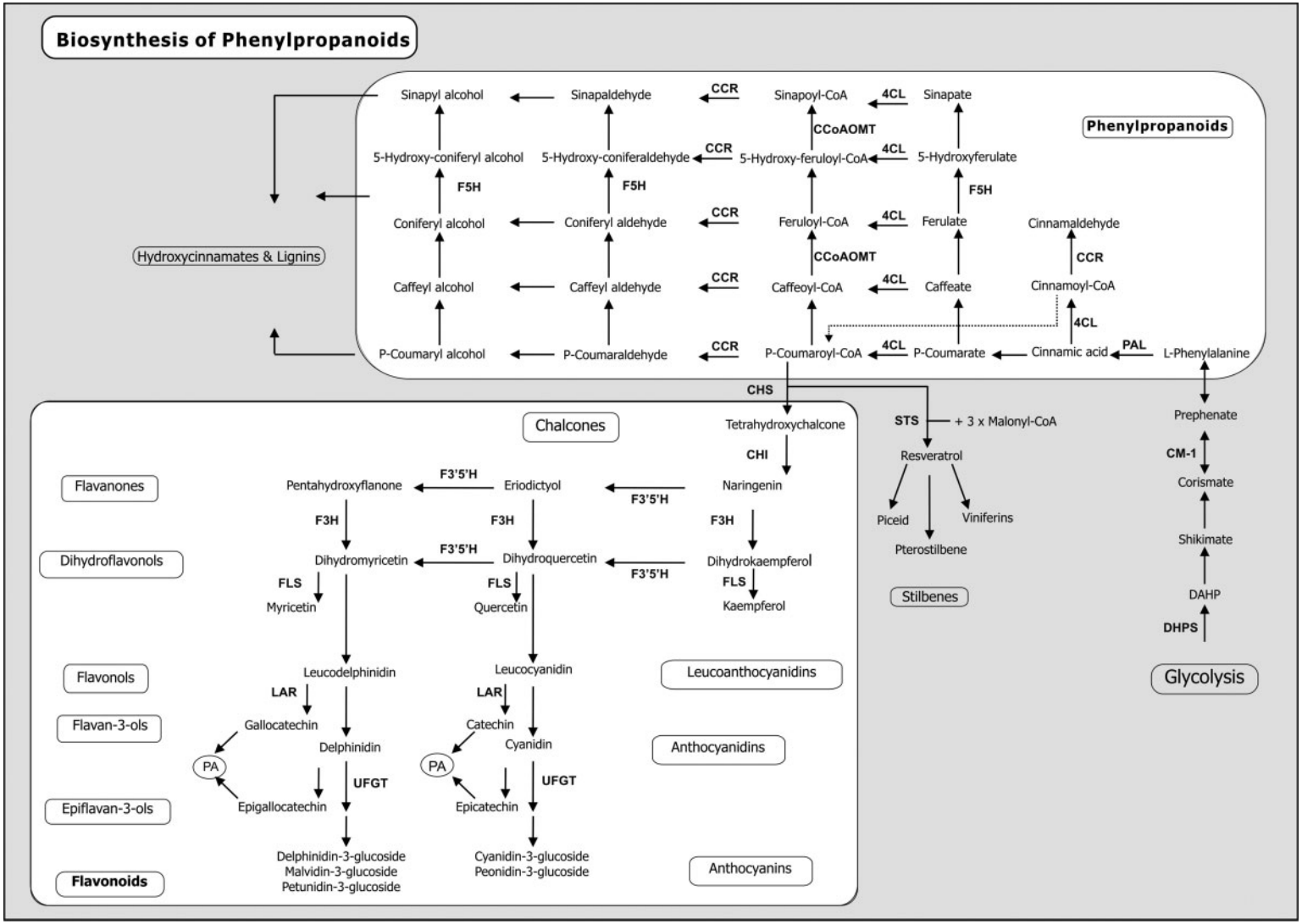

Figure 1. Schematic representation of the phenylpropanoid pathway derived from Velasco et al. (2007) and KEGG pathway from *Vitis vinifera* (wine grape). Note: The acronyms identify the enzymes whose transcripts were modulated in leaves of Pinot Noir grafted on 1103P and/or 101-14 grown on vineyard and sandy soils vs turf or within the comparison 1103P vs 101-14 rootstocks of plants grown on sandy soil. Abbreviations: 4CL, 4-Coumarate-CoA ligase; CCR, Cinnamoyl-CoA reductase; CHI, Chalcone-flavonone isomerise; CHS, Chalcone synthase; CM-1, Chorismate mutase 1; CCoAOMT, Caffeoyl-CoA-O-methyltransferase; DHPS, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase; F3'5'H, Flavonoid 3',5'-hydroxylase; F3H, Flavanone 3-hydroxylase; UFGT, UDP-glucose:flavonoid 3-O-glucosyltransferase, Flavonoid 3-O-glucosyltransferase; F5H, Ferulate 5-hydroxylase; FLS, Flavonol synthase; PAL, Phenylalanine ammonia-lyase; STS, Stilbene Synthase; LAR, Leucoanthocyanidin reductase. PA and DAHP are the acronyms of proanthocyanidins and 3-Dehyshikimate compounds, respectively.

Table 4. List of genes commonly modulated in three soil comparisons: vineyard vs turf on both rootstock combinations and sandy soil vs turf on 1103P rootstock.

Gene annotation	Probe sets	FC 1103P vineyard/ turf	FC 1103P sandy/ turf	FC 101-14 vineyard/ turf	BLASTN DFCI gene index release 7	E.C. number	UniProt
Pyrophosphate-dependent 6-phosphofructose-1-kinase (PPi-PFK)	1610956_at	2.5	2.43	2.15	TC105703	2.7.1.90	P21342
	1613360_at	2.22	2.18	2.72	TC105703		Q41140
Glyceraldehyde-3-phosphate dehydrogenase cytosolic (GAPDH)	1615814_at	15.22	13.42	9.12	TC105931	1.2.1.12	Q7FAH2
Phosphoglycerate kinase, cytosolic (PGK)	1610868_s_at	2.8	2.48	2	TC126068	2.7.2.3	Q42962
	1614662_at	2.84	2.04	2	TC126068		Q42962
Phosphoenolpyruvate carboxylase kinase 2 (PEPCK2)	1622074_at	7.85	3.96	9.46	TC132645	4.1.1.49	Q84JP7
Phosphoenolpyruvate carboxylase 1 (PEPC1)	1608100_at	8.77	5.67	6.21	TC105079	4.1.1.31	Q8S569
	1611103_at	2.55	/	2.36	TC114891		O23946
Phosphoglucomutase (PGM)	1607462_at	2.18	/	2.13	TC115213	5.4.2.2	Q9ZSQ4
Sucrose-phosphate synthase 1 (SPS1)	1614674_at	12	7.52	8.63	TC106827	2.4.1.14	Q5EEP9
L-idonate-2 dehydrogenase (L-IdnDH)	1622252_at	2.69	2.49	2.48	TC105024	1.1.1.128	Q9MBD7

Note: The variations in gene expression in vineyard soil vs turf are presented in the third column [the fold change (FC) is calculated as 1103P vineyard soil vs 1103P turf] and in the fifth column (FC calculated as 101-14 vineyard soil vs 101-14 turf), whereas the variations in gene expression in sandy soil vs turf are shown in the fourth column (FC calculated as 1103P sandy soil vs 1103P turf). The listed enzymes are displayed in Figure 2.

feruloyl-CoA to coniferaldehyde, and *p*-coumaroyl-CoA to *p*-coumaraldehyde (Figure 1).

CHS and *F3-O-GT* are part of the route toward the synthesis of many flavonoids and anthocyanin compounds (Winkel-Shirley 2001), and a variation in the expression level of these genes might suggest a regulation of the whole flavonoid pathway (Castellarin et al. 2007). A variation in the transcriptional profiling of key anthocyanin biosynthetic pathway genes was positively associated with the regulation of several probe sets corresponding to *GST*. The GSTs provide a binding activity required for the transport of anthocyanins from cytosol to the plant vacuole (Conn et al. 2008).

Genes regulated only in plant grafted on 1103P and grown on vineyard soil vs turf

The plants grafted on 1103P and grown on vineyard soil vs turf showed some specific features: first, a strong down-regulation on turf (corresponding to an up-regulation in vineyard soil) of the probe set corresponding to *VvMYBPA1* (FC 41.57), the MYB transcription factor controlling the PA biosynthesis (Bogs et al. 2007). It has been reported that grapevine leaves contained significant levels of PAs, although their composition differed from that in grape berry skins and seeds (Bogs et al. 2005, 2007). Moreover, the transcripts for *CHI*, for *F3H*, for *Flavonoid 3′,5′-hydroxylase* (F3′5′H), and for *FLS* were up-regulated, indicating that the route toward the synthesis of many flavonoid compounds was highly activated (the corresponding enzymes transform the flavanone compounds as naringenin, to 3-OH-flavanones as dihydrokaempferol and to flavonols such as kampferol) (Winkel-Shirley 2001) (Table 5). No regulation of DFR and LDOX and UFGT transcripts within the comparison has been observed, meaning that solely the higher part of the pathway should be influenced.

The second feature is a significant down-regulation on turf (corresponding to an up-regulation in vineyard soil) of the transcription of genes coding for enzymes involved in C-compound and carbohydrate metabolism (see Supplementary material 7: Carbohydrate metabolism glycolysis/gluconeogenesis TCA pathway-related ps). Besides the probe sets already reported (Table 4), additional sequences with annotations related to the C-compound metabolism were identified (Table 6). The probe sets corresponding to *ATP-citrate lyase* (ACL) and malate dehydrogenase (MDH) were up-regulated in plants grafted on rootstock 1103P and grown on vineyard soil. ACL catalyzes the reaction of citrate and CoA to form acetyl-CoA and OAA *via* malate (Fatland et al. 2002).

Cytosolic MDH catalyzes a reversible reaction between OAA and malate, and it is involved in the keeping of balance between these two intermediates.

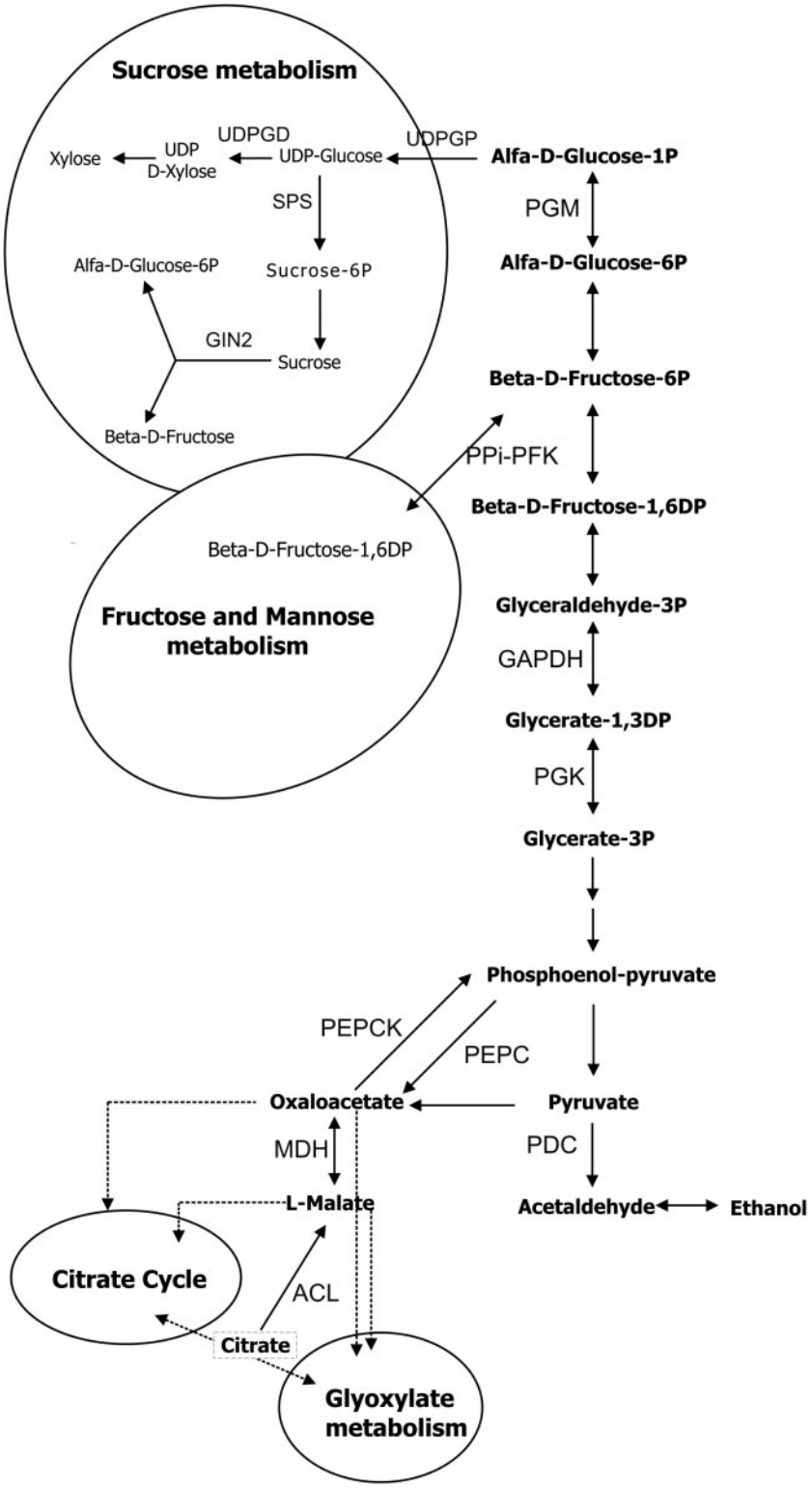

Figure 2. Schematic representation of the C-compound and carbohydrate metabolism derived from KEGG pathway from *Vitis vinifera* (wine grape). Note: The acronyms identify the enzymes whose transcripts were modulated in Pinot Noir grafted on 1103P and/or 101-14 grown on vineyard and sandy soils vs turf. Abbreviations: ACL, ATP-citrate lyase; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase cytosolic; GIN2, Vacuolar invertase 2; MDH, malate dehydrogenase; PDC, Pyruvate decarboxylase; PEPCK, Phosphoenolpyruvate carboxylase kinase; PGK, cytosolic phosphoglycerate kinase; PGM, Phosphoglucomutase; PEPC, Phosphoenolpyruvate carboxylase; PPi-PFK, Pyrophosphate-dependent 6-phosphofructose-1-kinase; SPS, Sucrose-phosphate synthase; UDPGD, UDP-glucuronate decarboxylase; UDPGP, UDP-glucose pyrophosphorylase.

Both cytosolic ACL and MDH enzymes lead to malate synthesis, one of the most prevalent acids in grape as well as in many other fruits (Sweetman et al. 2009). Moreover, the transcripts for *Pyruvate decarboxylase 1* (PDC1), *UDP-glucose pyrophosphorylase* (UDPGP), and for *Vacuolar invertase 2*

Table 5. List of genes involved in phenylpropanoid modulated in leaves of Pinot Noir grafted on 1103P rootstock grown on vineyard and sandy soils vs turf, and in leaves of Pinot Noir grafted on 101-14 rootstock grown on vineyard soil vs turf.

Gene annotation	Probe sets	FC 1103P vineyard/turf	FC 1103P sandy/turf	FC 101-14 vineyard/turf	BLASTN DFCI gene index release 7	E.C. number	UniProt
Phenylalanine ammonia-lyase (PAL)	1610206_at	2.47	2.45	/	TC108670		P45735
	1613113_at	6.02	3.79	/	TC110306	4.3.1.24	Q6UD65
	1619642_at	32.94	5.05	/	TC132375		Q94C45
Chorismate mutase (CM-1)	1611895_at	2.09	/	/	TC130364	5.4.99.5	P42738
4-Coumarate-CoA ligase (4CL)	1609307_at	2.26	/	/	TC119736	6.2.1.12	O24145
	1619320_at	2.82	/	/	TC106960		P31687
Caffeoyl-CoA-O-methyltransferase (CCoAOMT)	1614643_at	2.7	3.01	/	TC106122	2.1.1.104	Q43237
	1611897_s_at	4.03	4.4	/	TC125644		Q6J524
Cinnamoyl-CoA reductase (CCR)	1614423_at	6.11	5.25	/	TC123485	1.2.1.44	Q6DMZ8
Chalcone synthase (CHS)	1617019_at	14.69	6.26	/	TC105008	2.3.1.74	O80407
	1606663_at	30.28	6.24	/	TC114850		Q8W3P6
Chalcone-flavonone isomerase (CHI)	1615912_at	3	/	/	TC112432	5.5.1.6	P51117
Flavanone 3-hydroxylase (F3H)	1606435_at	3.68	/	/	TC105504		Q6R3N2
	1607607_s_at	4.26	/	/	TC105504	1.14.11.9	Q6R3N2
	1607739_at	/	/	9.21	TC137335		P41090
Flavonoid 3',5'-hydroxylase (F3'5'H)	1611829_at	3.28	/	/	TC128285	1.14.13.88	Q3HUM9
	1611847_at	/	/	6.59	TC111736		Q84NG3
Leucoanthocyanidin reductase (LAR)	1608212_at	/	2.17	/	TC105027	1.17.1.3	Q3S9L6
Flavonol 3-O-glucosyltransferase (F3-O-GT)	1615401_at	6.28	8.2	/	TC116915	2.4.1.91	Q40285
Anthocyanidin 3-O-glucosyltransferase (UFGT)	1617171_s_at	/	/	6.41	TC104973	2.4.1.115	O22303
	1619788_at	/	/	2.44	TC104973		Q9AVK6
Flavonol synthase (FLS)	1608791_at	3.51	/	/	TC105084	1.14.11.23	Q84TM1
3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DHPS)	1614440_at	/	2.4	3.4	TC107286	4.1.2.15	O24051
MYB-transcription factor protoanthocyanidins (VvMYBPA1)	1616094_at	41.57	/	/	TC107019	/	/
MYB-transcription factor for anthocyanins (VvMYBA1-A2)	1615798_at	/	/	2.26	TC105059	/	Q8L5P1
	1620959_s_at	/	/	12.58			Q6L973
Glutathione S-transferase (GST)	1614658_a_at		/	9.53	TC136061		Q56AY1
	1619917_s_at	/	/	9.20	TC136061		Q56AY1
	1612535_s_at	2.18	/	/	TC106802		Q76KW1
	1621636_s_at	2.11	2.41	/	TC111676	2.5.1.18	Q8GT24
	1609330_at	/	3.62	/	TC105720		Q6YEY5
	1616933_at	/	2.45	/	TC111676		Q948X4
	1622015_at	/	3.48	/	TC110049		Q4LAW7
	1610989_at	/	2.07	/	TC118101		Q03662

Note: The genes up-regulated in plants grafted on 1103P and grown on vineyard soil are listed in the third column [the fold change (FC) calculated as 1103P vineyard soil vs 1103P turf], whereas the genes up-regulated in sandy soil are listed in the fourth column (FC calculated as 1103P sandy soil vs 1103P turf), and the genes up-regulated in plants grafted on 101-14 and grown on vineyard soil are listed in the fifth column (FC calculated as 101-14 vineyard soil vs 101-14 turf). The listed enzymes are displayed in Figure 1.

Table 6. List of genes involved in C-compound and carbohydrate metabolism modulated in leaves of Pinot Noir grafted on 1103P rootstock grown on vineyard and sandy soils vs turf.

Gene annotation	Probe sets	FC 1103P vineyard/turf	FC 1103P sandy/turf	BLASTN DFCI gene index release 7	E.C. number	UniProt
Pyrophosphate-dependent 6-phosphofructose-1-kinase, beta subunit (PPi-PFK)	1619595_at	2.77	/	TC119136	2.7.1.90	Q9ZST3
UDP-glucose pyrophosphorylase (UDPGP)	1619491_s_at	2.2	/	TC109633	2.7.7.9	Q8W557
UDP-glucuronate decarboxylase (UDPGD)	1614795_at	2.41	/	TC129677	4.1.1.35	Q6T7C9
ATP-citrate lyase cytosolic, b-subunit (ACL)	1618362_s_at	2.16	/	TC114848	4.1.3.8	Q93YH3
Malate dehydrogenase cytosolic (MDH)	1607043_at	2.6	2.13	TC107076	1.1.1.37	Q9FT00
Pyruvate decarboxylase 1 (PDC1)	1611322_at	2.2	/	TC114657	4.1.1.1	Q9FVE1
Vacuolar invertase 2 (GIN2)	1612836_at	3.26	/	TC109507	3.2.1.26	Q9S943

Note: The genes up-regulated in plants grown on vineyard are listed in the third column [the fold change (FC) calculated as 1103P vineyard soil vs 1103P turf], whereas the genes up-regulated in plants grown on sandy soil are listed in the fourth column (FC calculated as 1103P sandy soil vs 1103P turf). The listed enzymes are displayed in Figure 2.

GIN2 were all up-regulated in 1103P grafted plants grown on vineyard soil vs turf. Pyruvate is located at the branching point between two alternative energy-producing processes: its conversion to acetyl-CoA by pyruvate dehydrogenase leads to respiration via the TCA cycle, whereas its conversion to acetaldehyde by pyruvate decarboxylase leads to fermentation. UDPGP catalyzes the reaction from α-D-Glucose-1P to UDP-glucose, the substrate for sucrose biosynthesis, while GIN2, the invertase, is responsible for the conversion of sucrose into glucose and fructose (Table 6, Figure 2).

Genes regulated only in plant grafted on 101-14 and grown on vineyard soil vs turf

When the vineyard soil vs turf comparison was performed with transcriptomic data of plants grafted on rootstock 101-14, five genes annotated as related to flavonoid biosynthesis were identified as down-regulated in turf (vice versa up-regulated in vineyard soil) (Table 5): *F3H, F3′5′H, Anthocyanidin 3-O-glucosyltransferase*, a UFGT, and the regulators of the anthocyanin synthesis *VvMYBA1* and *VvMYBA2* (activators of UFGT), involved in the last steps in anthocyanin biosynthesis. Moreover, the steady-state mRNA level of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase, a key enzyme of the shikimate and phenylpropanoid pathways, increased more in plants grown on vineyard soil than turf (Table 5, Figure 1).

Abiotic-stress-related genes

Some abiotic-stress-related genes, including *heat shock proteins* (HSPs), *heat shock transcription factor* (HSTF30), *Galactinol synthase (GolS), and ascorbate peroxidase 1(APX1)* were up-regulated in leaves of Pinot Noir grafted on 1103P rootstock grown on vineyard soil in comparison with the same plants grown on turf. In addition, six stress-related genes were up-regulated (and two down-regulated) in plants grafted on 1103P compared to plants grafted on 101-14 (Table 7) when grown on sandy soil (see Supplementary material 8: Drought and ABA pathway-related ps). The small HSPs are ubiquitous in terms of cellular localization and act as molecular chaperones capturing unfolding proteins to form stable complexes (Nakamoto & Vígh 2007). Nevertheless, the HSP family also contains members essential for normal growth and development (Kotak et al. 2007). GolS produces galactinol from UDP-galactose, and the up-regulation of this gene can be related to the well-known accumulation of oligosaccharides in response to different abiotic stress conditions (Gupta & Kaur 2005).

Validation of array data by qRT-PCR

To validate the array data, 16 probe sets representing relevant genes involved in pathways modulated by soil and rootstock and detailed in Materials and Methods were subjected to qRT-PCR analysis. The ubiquitin gene corresponding to 1616334_a_at probe set was selected as reference. The results are summarized in Supplementary material 2: Gene expression validation by qRT-PCR, where for each probe set the microarray value was compared with the corresponding qRT-PCR data. The qRT-PCR expression values of all selected genes were in good agreement with the microarray values (Pearson's correlation coefficient 0.8). The validation experiment confirms that the regulation of the phenylpropanoid pathway and of the carbohydrate metabolism is affected by signals from rootstock and/or soil.

Table 7. List of abiotic stress-related genes modulated in response to soil and rootstock effects.

Gene annotation	Probe sets	FC 1103P vineyard/ turf	FC 1103P sandy/ turf	FC 101-14 vineyard/ turf	FC sandy soil 1103P/ 101-14	BLASTN DFCI gene index release 7	UniProt
Heat shock protein (HSP18.2)	1612385_at	11.75	3.25	/	−3.57	TC133866	Q9SYV0
Heat shock protein (HSP60)	1609502_at	2.32	/	/	/	TC136835	Q05046
Heat shock protein (HSP70)	1609949_at	2.27	/	/	/	TC110606	Q5QHT3
Heat shock protein (HSP18.6)	1619616_at	3.3	2.46	/	/	TC112800	Q39929
Heat shock transcription factor (HSTF30)	1610122_at	2.94	/	/	/	TC105891	P41152
Heat shock protein (HSP22)	1620348_at	2.23	/	/	/	TC105973	Q96331
Tubulin beta-1 (TUB1)	1616815_at	3.92	/	/	−2.72	TC122119	P45960
Galactinol synthase (GolS)	1609808_at	3.23	/	/	/	TC110130	Q9XEJ7
	1621902_at	/	/	3.45	/	TC110130	Q9XEJ7
Ascorbate peroxidase 1, cytosolic (APX1)	1606498_s_at	2.14	/	/	/	TC129540	O48919
Abscisic acid responsive elements-binding factor (AREB1)	1619029_at	2.11	/	/	/	TC108628	Q9M4H1
9-*cis*-epoxycarotenoid dioxygenase 1 (NCED)	1606788_s_at	/	/	2.56	/	TC104874	Q5SGD1
Early responsive to dehydration (ERD15)	1615249_at	2.06	/	/	/	TC107435	Q9LKW3
Dicyanin	1621220_at	2.38	2.56	/	3.01	TC110284	Q9M510
Tubulin beta-8 (TUB8)	1620596_at	2.08	/	/	/	TC109303	P3783
Dehydrin 1b	1621592_s_at	/	/	−2.78	/	TC111806	Q3ZNL4
Anionic peroxidase precursor	1609321_at	/	/	/	2.98	TC113237	Q43032
Salt tolerance zinc finger (STZ, ZAT10)	1609107_at	/	/	/	2.4	TC124693	Q4AEC3
Thioredoxin protein (F3M18.8)	1616703_at	/	/	/	2.88	TC111873	Q9SGP6
Harpin inducing protein (Hin1)	1611643_at	/	/	/	3.11	TC109529	Q75QH3
Short-chain alcohol dehydrogenase-like	1608541_s_at	/	/	/	2.1	TC130642	Q8LFZ4

Note: Transcripts up-regulated in leaves of Pinot Noir grafted on 1103P grown on vineyard soil compared to the same plants grown on turf are listed in the third column [the fold change (FC) calculated as 1103P vineyard soil vs 1103P turf], whereas the genes up-regulated in plants grafted on 1103P and grown on sandy soil compared to the same plants grown on turf are listed in the fourth column (FC calculated as 1103P sandy soil vs 1103P turf). Transcripts up-regulated in leaves of Pinot Noir grafted on 101-14 grown on vineyard compared to the same plants grown on turf are listed in the fifth column (FC calculated as 101-14 vineyard soil vs 101-14 turf), whereas the genes up-regulated in plants grafted on 1103P compared to the same plants grafted on 101-14 when grown on sandy soil are listed in the sixth column (FC calculated as sandy soil 1103P vs 101-14).

Discussion

Vineyard soil composition and rootstocks are important factors for vine development and, consequently, for wine characteristics. A long-lasting debate on the influence of soil and rootstock on grapevine growth was held (Mackenzie & Christy 2005; Andrés-de-Prado et al. 2007; Sampaio 2007; Trought et al. 2008; Ubalde et al. 2010). Nevertheless, no studies so far have addressed the effects of these factors on the transcriptome of the scion. The experimental design of the present work has allowed us to investigate the effects of two rootstocks and three substrates on the leaf transcriptome of the scion of Pinot Noir. The 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 vineyard and sandy soils.

Sandy soil represents a non-optimal growing substrate, because it is devoid of nutrients (Table 1) and it has a low water-holding capacity (Creasy & Creasy 2009). This is a condition that can lead to some degree of drought stress, despite all the plants irrigated to get to the field capacity. On this substrate, a key feature of the response induced by the vigorous 1103P compared to the low-vigor 101-14 rootstock was represented by a general up-regulation of the phenylpropanoid metabolism in the scions grafted on 1103P. 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 (Tables 3 and 5).

The up-regulation of the transcripts coding for CCoAOMT and FH5 is expected to promote the lignin biosynthesis. Lignin represents a physical barrier in response to microbial attacks and functions in water transport as a hydrophobic constituent of vascular phloem and xylem cells (Ferrer et al. 2008). The induction of mRNAs coding for STSs should promote the plant response to biotic stresses as suggested by the repellent role of phytoalexin in the defensive responses to infections (Ferrer et al. 2008). In addition, the up-regulation of mRNAs for F3H and F3-O-GT and VlMYBs drives the pathway toward the formation of anthocyanidins and anthocyanins, respectively, in the flavonoid biosynthesis route (Table 3).

Overall, 1103P rootstock promoted particularly on vineyard and sandy soils, 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. These results suggest that 1103P confers to the Pinot Noir a broad plant resistance, a consideration in agreement with the previous findings. Treutter (2006) has pointed out the role of flavonoids as 'preformed' compounds constitutively synthesized during the normal development of plant tissue and 'induced' compounds synthesized by plants in response to physical injury, infection, or stress. Satisha et al. (2007) have observed that the rootstocks belonging to *V. berlandierii* × *V. rupestris*, such as 1103P, recorded the highest values for total phenols, total proteins, flavonoids, and flavon-3-ols. This may help the rootstock in overcoming the incidence of important grape diseases and lead to a better rooting percentage. Though the study had not covered the influence of the effect of rootstocks on the physiology and biochemistry of scions after grafting, the inherent capacity of the rootstocks of having a positive influence on these aspects after grafting is well demonstrated by several reports describing the effect of rootstocks on the biochemical composition of the scion in various species, such as apple (Brown et al. 1985), soybean (Carver et al. 1987), peach (Giorgi et al. 2005), and grape (Ruhl et al. 1988; Ezzahouani & Williams 1995; Gawel et al. 2000; Reynolds & Wardle 2001; Main et al. 2002; Ollat et al. 2003). In *Hevea* and watermelon, the rootstocks have a deep influence on the biochemical composition of the leaves, particularly in terms of enzymes, reducing sugars, phenols, and amino acids (Sobhana et al. 2001; Evrenoso et al. 2010). The synthesis of stilbenes can be constitutive or induced by biotic and abiotic elicitors. Oligomers of resveratrol are present as constitutive substances in the lignified organs of grapevine, such as roots, stems, canes, and seeds. Upon fungal infection in leaf, *trans*-resveratrol is synthesized and converted into more toxic derivatives (viniferins), although a comprehensive analysis of the constitutive accumulation of resveratrol in healthy grape is still lacking to date (Jeandet et al. 2002; Gatto et al. 2008; Deluc et al. 2011; Malacarne et al. 2011).

A main pathway regulated by rootstock and soil factors is carbohydrate metabolism. Total soluble carbohydrates (glucose, fructose, and sucrose) are estimated to represent approximately 70% of the osmotically active solutes in young grapevine leaves (Patakas 2000), but few data concerning the impact of different substrates on vineyards development and particularly on leaves have been published. Van Leeuwen et al. have verified that berry weight, berry sugar, and anthocyanin concentration are mainly influenced by the soil type (Van Leeuwen et al. 2004). The tartaric acid with malate is the most abundant organic acid in grapevine, and the tartrate content increases very rapidly during leaf elongation and in preveraison of the berries (Kliewer & Nassar 1966; DeBolt et al. 2006). l-idonate dehydrogenase is the key enzyme involved in tartaric acid biosynthesis. Its corresponding transcript was found to increase differentially in Pinot Noir scions grown on vineyard and on sandy soils (Table 4).

Turf soil in plants grafted on 1103P rootstock led to a down-regulation of the *ACL* and *MDH* transcripts in comparison with plants grown on vineyard and on sandy soils (Table 6). ACL is a cytosolic enzyme, implying that it generates a cytosolic pool of acetyl-CoA required for the biosynthesis of a plethora of phytochemicals. Many of these compounds are important for plant growth and responses to environmental cues (Fatland et al. 2005). Both ACL and MDH enzymes are involved in malate synthesis that is the most prevalent organic acid together with tartaric acid, molecules related to aroma and taste of the wine. Both leaves and immature green berries are able to form malic and tartaric acids (Kliewer & Nassar 1966; Conde et al. 2007).

Some stress-related genes have been detected among the genes modulated in the experiments (Table 7). These findings suggest that a transcriptome response to abiotic stress, particularly drought, could be ongoing in some conditions due to the limited water availability of some soil types. Nevertheless, the molecular response to water stress in vegetative tissue affects a plethora of genes responsible of several physiological changes, such as an increase of ABA concentrations correlated with the transcript abundance of *VvNCED1* (Soar et al. 2004), a reduction in stomatal conductance and photosynthesis by photoinhibition of photosystem II, the aquaporin gene expression, the solutes accumulation for osmotic adjustment, fatty acid, and ROS metabolism, and transcription factors activation, i.e. for DREB or ethylene response factors (Cramer et al. 2007; Cramer 2010). In this work the number of modulated transcripts with a potential drought-related involvement is low and should not be considered a drought stress response; nevertheless, it cannot be excluded

that a mild drought stress was responsible for the modulation of some of the probe set identified.

The array analysis highlighted a differential activation of genes related to the phenylpropanoid pathway, particularly to stilbenoid and flavonoid biosynthesis, to the carbohydrate and energetic pathways as well to the stress-responsive mechanisms. These findings indicate that soil and rootstock have an influence on the transcriptome of the leaves of scion cv. Pinot Noir. They also give a contribution to provide the molecular bases to explain the effects of soils and rootstocks on the grapevine grown.

Acknowledgements

This work was financially supported by the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) VIGNETO and RINGO projects.

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