UNRAVELLING THE REGULATORY NETWORK PUTATIVELY CONTROLLING FLAVONOID BIOSYNTHESIS IN GRAPEVINE

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UNRAVELLING THE REGULATORY NETWORK PUTATIVELY CONTROLLING FLAVONOID BIOSYNTHESIS IN GRAPEVINE

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Chapter 1

INTRODUCTION

The flavonoid biosynthetic pathway

Flavonoids are secondary metabolites that are widely accumulated in plant kingdom and involved in several aspects of plant development and defense. The flavonoid family encompasses at least 6000 molecules divided in chalcones, flavones, flavonols, flavandiols, anthocyanins, proanthocyanidins (PA) and aurones. All these compounds exhibit a wide range of biological functions in plants.

Flavonoids play a protective role against many abiotic stresses as oxidative damage or the UV-B rays (Sarma AD, 1999; Harborne and Williams, 2000; Dixon *et al.*, 2002; Dixon, 2005; Koes *et al.*, 2005; Aron and Kennedy, 2008; Albert *et al.*, 2009) and also in the interaction between plants and animals: for example, proanthocyanidins accumulated in leaves determine the bitter taste, preventing feeding by herbivores (Harborne and Williams, 2000; Aron and Kennedy, 2008). They are also involved in the control of physiological traits of plant development as pollen fertility, modulation of auxin transport, pollinator attraction, seed dispersal and seed coat-imposed dormancy (Winkel-Shirley, 2000; Brown *et al.*, 2001; Debeaujon *et al.*, 2001; Debeaujon *et al.*, 2003; Lepiniec *et al.*, 2006; Peer and Murphy, 2007; Thompson *et al.*, 2010).

In grapevine, flavonoids play a pivotal role contributing to the organoleptic qualities of the fruits and the wine. In particular, anthocyanins, accumulated in the skin of the berries during the ripening, are responsible for the colour of red wines. PA are stored mainly in the berry skin and in seed tissues and are important for the structure and mouth feel of white and red wines. Flavonols, present in the skin, contribute to the colour of wine interacting with the other compounds. Because of their importance for wine quality and conservation and their known beneficial effects to human health, lately many researchers focused their attention on the flavonoid biosynthetic pathway and their regulation in grapevine (Boss *et al.*, 1996; Kobayashi *et al.*, 2002; Bogs *et al.*, 2005; Bogs *et al.*, 2006; Deluc *et al.*, 2006; Bogs *et al.*, 2007; Walker *et al.*, 2007; Deluc *et al.*, 2008; Czemmel *et al.*, 2009; Terrier *et al.*, 2009).

Regulation of the flavonoid biosynthetic pathway

Flavonoids are synthesized from the action of multiple enzymes via the general phenylpropanoid pathway (Figure 1) that provides also precursors for the production of other secondary metabolits such as lignin. The flavonoid biosynthesis has been largely studied in many species, where the majority of the enzymatic reactions is conserved despite they display different flavonoid profiles. Although they derive from the same biosynthetic pathway, flavonoids serves different biological functions in specific organs and tissues, depending on the developmental stage and the environmental conditions. Thus, the synthesis of each group is finely regulated ensuring that the appropriate compounds are produced when and where required. One of the mechanism that finely regulates this biosynthetic pathway consists of a complex conserved in many species that includes two transcription factors (TFs) belonging to MYB and bHLH families (Holton and Cornish, 1995) and a WD40 regulatory protein.

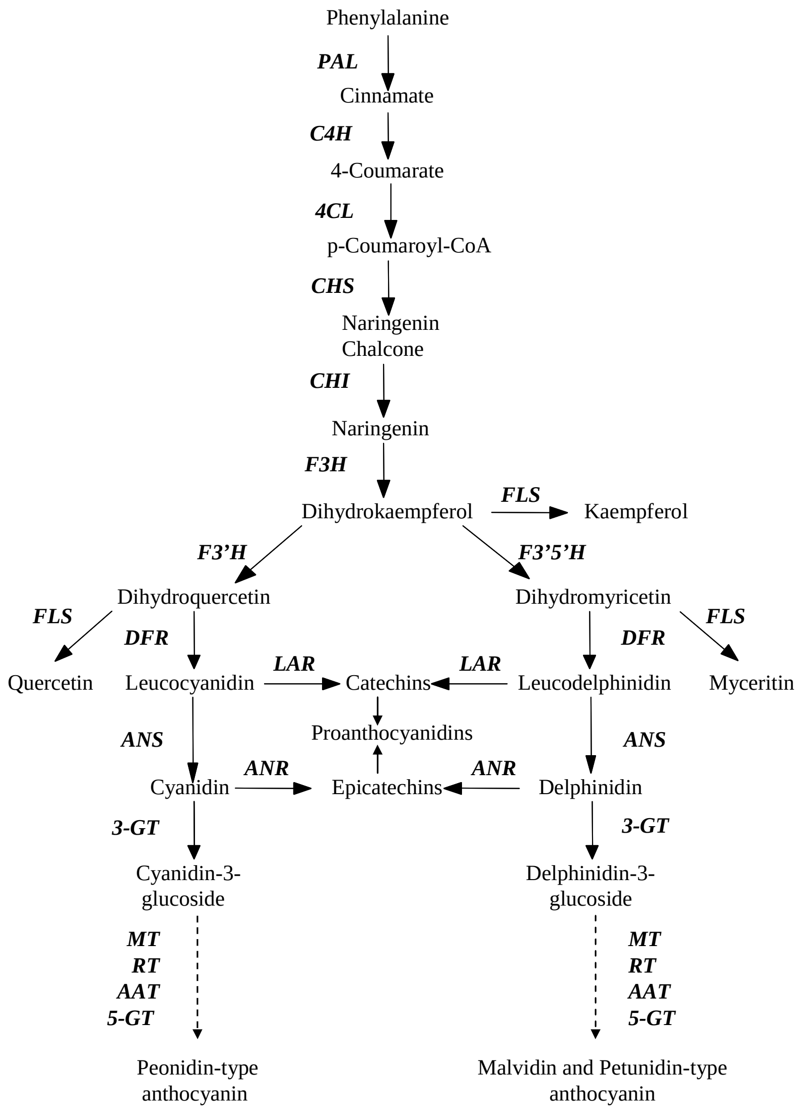

Figure 1. Scheme of the flavonoid pathway leading to synthesis of anthocyanins, flavonols, and PAs. The enzymes involved in the pathway are shown as follows: *PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA-ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; FLS, flavonol synthase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavonoid 3'5'-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase; 3-GT, flavonoid 3-O-glucosyltransferase; MT, methyltransferase, RT, flavonoid 3-O-glucoside-rhamnosyltransferase; AAT, anthocyanin acyltransferase; 5-GT, flavonoid 5-glucosyltransferase.*

In the complex the MYB factor is responsible of the recognition and binding of specific *cis*-elements in the promoter sequences of the structural genes with the N-terminal region, and it regulates the gene expression through activation or repression domains at the C-terminus. For this reason, the MYB protein plays a fundamental role driving the complex and determining the specific subset of genes to be regulated. The bHLH proteins can also bind to DNA with the bHLH domain and directly interact with the MYB factor and the WD40 protein, which is thought to confer a docking platform for the MYB-bHLH interaction.

The requirement of this transcriptional complex for the regulation of the flavonoid pathway has been established in *Petunia hybrida,* where the WD40 protein ANTHOCYANIN 11 (AN11) and the two bHLH proteins ANTHOCYANIN 1 (AN1) and JAF13 interact with the MYB protein ANTHOCYANIN 2 (AN2) to regulate anthocyanin production in petal epidermis (Quattrocchio *et al.*, 1999; Spelt *et al.*, 2000). Other information were provided by studies of mutants in Arabidopsis, where the complex have been shown to regulate specifically anthocyanin and proanthocyanidin biosynthesis depending on the MYB factor recruited by the complex. The WD40 protein TRANSPARENT TESTA GLABRA 1 (TTG1) interacts with the partially redundant bHLHs GLABRA 3 (GL3) and ENHANCER OF GLABRA 3 (EGL3) and the MYB proteins PRODUCTION OF ANTHOCYANIN PIGMENT 1 AND 2 (PAP1 and PAP2) to induce anthocyanin accumulation in hypocotyls (Borevitz *et al.*, 2000). A similar complex consisting of TTG1 plus the bHLH TT8 and the partially redundant MYB proteins MYB5 and TT2 regulates tannin production in seed coat (Li *et al.*, 2009).

Other regulative functions of the WD40-bHLH-MYB complex

In addition to the flavonoid pathway, the regulatory complex controls other cellular differentiation pathways leading to the definition of diverse epidermal cell fates. In fact, the presence of many transcription factors could generate multiple combinations of protein interaction in the regulatory complex, resulting in recognition of various *cis*-regulatory elements in the target genes. One typical example is represented by *Arabidopsis thaliana*, where the regulatory complex formed by the WD40 protein AtTTG1 in combination with different sets of bHLH and MYB proteins controls anthocyanin and proanthocyanidin (PA) biosynthesis, trichome development, hairy root formation and mucilage production (Lee and Schiefelbein, 1999; Walker *et al.*, 1999; Borevitz *et al.*, 2000; Kirik *et al.*, 2005; Gonzalez *et al.*, 2009; Li *et al.*, 2009). In petunia the presence of specific MYB factors determines which process is regulated: as mentioned above, the MYB protein PhAN2 guides the complex in the regulation of the anthocyanin synthesis in the epidermal cells of the petal (Quattrocchio *et al.*, 1999). A different transcription factor PhPH4 is instead responsible of the acidification of the vacuoles where the pigments are stored (Quattrocchio *et al.*, 2006).

Another aspect that contributes in providing more variability of the regulated processes is the multitude of additional regulators acting in concert or downstream the complex. This includes transcription factors with a MADS box (Nesi *et al.*, 2002; Qi *et al.*, 2011), a Zn-finger (Sagasser *et al.*, 2002), or a WRKY domain (Johnson *et al.*, 2002). Among these co-regulators, the Arabidopsis WRKY transcription factor TRANSPARENT TESTA GLABRA 2 (TTG2) has been reported to be involved in trichome development, PA and mucilage production in seed coat. It was demonstrated that AtTTG2 is under the control of the MYB proteins AtGL1, AtMYB5 and AtTT2 in the AtTTG1-regulatory networks (Johnson *et al.*, 2002; Ishida *et al.*, 2007). Another regulator belonging to the WRKY family is the petunia PH3 that acts downstream and in concert with the regulatory complex directed by the MYB protein PhPH4 in the regulation of the vacuolar acidification (Quattrocchio *et al.*, submitted).

The regulatory complex in grapevine

As in other species, also in grapevine the presence of the WD40–bHLH–MYB complex is required for the regulation of the flavonoid biosynthetic pathway (Deluc *et al.*, 2008; Hichri *et al.*, 2010). As putative members of the complex, recent studies identified two bHLH transcription factors, VvMYC1 and VvMYCA1, and the regulatory protein VvWD1 (Hichri *et al.*, 2010; Matus *et al.*, 2010). More detailed information is available about the MYB proteins, that are the responsible of specific branches of the flavonoid pathway that the regulatory complex controls. For example, VvMYBA1 and VvMYBA2 are responsible for the activation of UDP-glucose: flavonoid 3-O-glucosyltransferase (UFGT) during anthocyanin synthesis (Kobayashi *et al.*, 2002; Kobayashi *et al.*, 2004; Walker *et al.*, 2007) while VvMYBPA1 and VvMYBPA2 regulate *VvLAR1* and *VvANR* expression specific of the proanthocyanidin branch in addition to the general flavonoid pathway (Bogs *et al.*, 2007; Terrier *et al.*, 2009). Recently, VvMYB5a and VvMYB5b have been identified as putative regulators of the general steps of the flavonoid pathway (Deluc *et al.*, 2006; Deluc *et al.*, 2008); however, their functions remain to be elucidated.

Because of the high similarity, VvMYB5a and VvMYB5b belong to a cluster that includes other MYB proteins of various species, as AtMYB5 and PhPH4, that are involved in the control of different physiological and developmental processes.

As mentioned above, the Arabidopsis MYB5 regulate the WRKY transcription factor AtTTG2 in the network that leads to the control of the trichome development, PA and mucilage production in seed coat (Johnson *et al.*, 2002; Ishida *et al.*, 2007). A similar regulative mechanism is observed in petunia, where the MYB factor PhPH4 controls the vacuolar pH through the activation of the WRKY transcription factor PhPH3, the functional ortholog to AtTTG2 (Quattrocchio *et al.*, submitted). Despite the diversity of the controlled processes, the high sequence similarity between the MYB factors, AtMYB5 and PhPH4, and between the downstream WRKY regulators, AtTTG2 and PhPH3, suggests that this regulatory network may be conserved also in other species. Despite the grape MYB factors orthologs to Arabidopsis MYB5 and petunia PH4 have been already identified in VvMYB5a and VvMYB5b (Deluc *et al.*, 2006; Deluc *et al.*, 2008), no intermediate regulator downstream VvMYB5a and VvMYB5b was previously described.

Outline of the thesis

The goal of this PhD research project was to determine which are the biological processes regulated by the regulatory complex driven by VvMYB5a and VvMYB5b in grapevine. As first, we performed functional characterization of VvMYB5a and VvMYB5b using different approaches as complementation analyses in petunia mutants and stable transformation of *Vitis vinifera* in order to understand which are the functions in grapevine. We verified the physical interactions between VvMYB5a, VvMYB5b and the other bHLH and WD40 members. We also identified putative downstream genes in the network directed by the complex to demonstrate that this regulatory pathway, well described in Arabidopsis and petunia, is conserved also in grapevine.

Chapter 2 describes the heterologous expression of *VvMYB5a* and *VvMYB5b* in some well characterized petunia pH/anthocyanin regulatory mutants demonstrating that VvMYB5a and VvMYB5b are involved in the regulation of the vacuolar acidification and partially also in the activation of the flavonoid pathway in the epidermal cells of petals in petunia.

Chapter 3 reports the functional characterization of VvMYB5a and VvMYB5b in grapevine. As first, we performed ectopic expression of *VvMYB5a* in grape hairy roots. Transcriptomic analysis confirmed its role in the flavonoid pathway and provided indications of its involvement in the control of many other biological processes. Secondly, *Vitis vinifera* was stably transformed with *Agrobacterium tumefaciens* to silence and overexpress both *VvMYB5a* and *VvMYB5b*. The phenotypic characterization of the transgenic plants revealed their specific ability in the regulation of proanthocyanidins and flavonol branches in addition to the flavonoid general pathway. We also provide evidence of their involvement in other biological processes as mesophyll organization in leaves and the control of stress-related genes.

In **chapter 4**, we describe the isolation of VvWRKY19, a WRKY transcription factor of *Vitis vinifera*, that is highly similar to AtTTG2 and PhPH3. Heterologous expression in petunia *ph3* mutant demonstrated that VvWRKY19 and PH3 are functionally homologs and that VvWRKY19 can regulate the vacuolar acidification pathway in petunia. We suggest that in grapevine VvWRKY19 is an intermediate regulator acting downstream VvMYB5a and VvMYB5b and in concert with the regulatory complex. A microarray analysis on transgenic grapevines silenced for *VvWRKY19* expression provided information on the putative biological processes that this transcription factor regulates. In conclusion, we discuss the metabolic pathways putatively regulated by VvWRKY19, VvMYB5a and VvMYB5b in grapevine.

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Chapter 2

Exploring the function of the grape regulators VvMYB5a and VvMYB5b by heterologous expression in anthocyanin and vacuolar pH petunia mutants

ABSTRACT

Flavonoids are secondary metabolites accumulated during grape berry development and important for grape and wine quality. Despite the flavonoid biosynthetic pathway has been well studied, little is known about the regulative mechanism that controls it. Recent studies suggest that two MYB transcription factors, VvMYB5a and VvMYB5b, are responsible for the regulation of the early flavonoid structural genes in different stages of berry development (Deluc *et al.*, 2006; Deluc *et al.*, 2008).

We used functional complementation analyses of some well characterized Petunia anthocyanin / pH regulatory mutants to gain information about the roles of VvMYB5a, VvMYB5b in *Vitis vinifera* and to compare them to the function of the anthocyanin regulator VvMYBA1.

In petunia the mutation of the ortholog of *VvMYB5a* and *VvMYB5b* (*PhPH4*) and of *VvMYBA1* (*PhAN2*) results in the increase of vacuolar pH and strong reduction of anthocyanin content of petals, respectively. The coding sequence of *VvMYB5a*, *VvMYB5b* and *VvMYBA1* was fused to the constitutive promoter 35S and transformed into *ph4* and *an2* petunia mutant lines. Analyses of transgenic plants revealed full complementation phenotypes. Deep analyses of pigment profile, vacuolar pH and expression of structural genes confirmed that restored phenotypes were attributable to an activation of target genes belonging to vacuolar acidification and anthocyanin pathways. Moreover, specific and/or partially overlapping effects are assessed. Microarray analyses of the transgenic lines gave insights about the possible redundant roles of these MYB transcription factors in grape.

INTRODUCTION

The colour of most flowers and fruits depends on the accumulation of anthocyanin in the vacuoles of epidermal cells. Chemical modifications of these pigments as well as the presence of other specific colourless compounds can influence the organ pigmentation. Another factor that strongly affects the colour is the pH of the vacuolar lumen where the anthocyanins are stored. The vacuolar pH influences the absoption spectrum of the anthocyanins and consequently the pigmentation of the cells. When the vacuolar lumen is weakly acidic (pH=5), the anthocyanins appear reddish compared to pigments contained in less acidic vacuoles.

The wealth of knowledge accumulated in the last several decades about the flavonoid biosynthetic pathway has made Petunia the species of choice for the study of plant tissue pigmentation (Holton and Cornish, 1995; van Houwelingen, 1998). In this species, the anthocyanin synthesis takes place at particular stages of development and requires a coordinate regulation of the flavonoid structural genes. A transcriptional complex well conserved in many species is responsible for the regulation of this pathway (Koes, 2005). In particular, in petunia this complex formed by two BHLH factors ANTHOCYANIN 1 (PhAN1) and PhJAF13, a WD40 protein ANTHOCYANIN 11 (PhAN11) and a MYB transcription factor ANTHOCYANIN 2 (PhAN2) control the late structural genes *CHSj*, *DFRa*, *ART*, *AMT* and *AN9* (Beld, 1989; Quattrocchio, 1993), while the early biosynthetic genes *CHSa*, *CHIa*, and *F3H* are expressed independently. Recent studies revealed that the regulation of the anthocyanin production is tightly linked to that of vacuolar acidification. In fact, both processes are under control of the same regulatory proteins (PhAN1, PhJAF13 and PhAN11) which interact with multiple possible MYB partners giving flexibility to the system and integrating the two pathways in one regulatory network. A specific MYB transcription factor PhPH4 is recruited by the complex, alternatively to PhAN2, to activate vacuolar acidification. PhPH4 was previously identified among seven loci (named *PH1* to *PH7*) that, when mutated, cause a more bluish flower color and an increase in the pH of crude petal extracts (Wiering, 1974; de Vlaming, 1982; van

Houwelingen, 1998). The genes regulated by the complex driven by PhPH4 are the intermediate regulator *PhPH3*, a WRKY transcription factor, and the pumps *PhPH1* and *PhPH5*. Recent studies showed that PhPH5 is a P_{3A}-ATPase proton pump localized on the tonoplast (Verweij *et al.*, 2008) while PhPH1 is a P_{3B}-ATPase also required for vacuolar pH regulation in petal flowers (Provenzano, 2011). Another gene down-regulated in *ph4* mutant line and possibly involved in the acidification pathway is *PhCAC 16.5*, a putative cysteine proteinase, whose function is still unclear (Quattrocchio *et al.*, 2006).

Recently, it was demonstrated that PhPH4, PhPH3 and PhAN1 are responsible also for the activation of a set of genes for the cellular transport vesicles (Faraco, 2011). They regulate the formation of an intermediate cellular compartment that deliveries proteins to the central vacuoles through a fusion process mediated by PhPH1 and PhPH5 proteins.

Similarly to petunia petals, grape berry colour depends on the accumulation of anthocyanins into vacuoles of epidermal cells during ripening. Because colour of grape berries is a fundamental parameter for fruit quality and the wine, understanding the regulatory mechanisms of these pathways can offer the possibility to control berry anthocyanin content in the vineyard and to improve grape and wine quality. Several grape anthocyanin structural genes have been well characterized (Boss *et al.*, 1996). Some data indicate that the grape regulators of anthocyanin biosynthesis are homologous to those found in petunia. For example, the transcription factors VvMYBA1 and VvMYBA2, specific regulators of the anthocyanin metabolic pathway in berries are homologs to petunia AN2 (Kobayashi *et al.*, 2002; Kobayashi *et al.*, 2004; Walker *et al.*, 2007). In addition, two proteins, VvMYB5a and VvMYB5b, have been recently identified as possible regulators of the flavonoid biosynthetic pathway (Deluc *et al.*, 2006; Deluc *et al.*, 2008). Both proteins belong to a small cluster of R2R3-MYB transcription factors involved in the control of various physiological and developmental processes. They appear to be homologue to petunia PH4, involved in regulation of vacuolar acidification (Quattrocchio *et al.*, 2006) and to Arabidopsis MYB5 that controls seed coat differentiation, trichome development, tannin synthesis and mucilage production (Gonzalez *et al.*, 2009; Li *et al.*, 2009). When expressed in tobacco, they impact the metabolism of anthocyanins, flavonols, tannins and lignins in reproductive organs. Moreover, transient expression in grape cells showed that VvMYB5a and VvMYB5b can directly activate the promoters of several genes of the flavonoid pathway. In berries, expression of *VvMYB5b* is high in the skin tissues in early stages of development and increases further during ripening (Deluc *et al.*, 2008). In contrast, *VvMYB5a* is almost exclusively expressed before veraison (Deluc *et al.*, 2006). These differential expression profiles suggest that VvMYB5a may be particularly involved in the control of proanthocyanidin synthesis in skin and seed tissues before veraison, whereas VvMYB5b may participate in the regulation of both proanthocyanidin and anthocyanin biosynthesis throughout berry development. Despite the information available, their functional characterization remains partial and requires further study.

To obtain insights about the roles of VvMYB5a and VvMYB5b, here we perform complementation analyses using petunia regulatory mutants impaired in pigment accumulation and vacuolar acidification in flower epidermis, proven to be a valid tool for gene functional studies.

MATERIAL AND METHODS

Plant material and growth conditions

Petunia plants derived from the collection of Amsterdam University were grown under normal greenhouse conditions. The *ph4* mutated line I11 used for complementation studies is heterozygous for the allele $ph4^{V64}$ harboring the transposon *dTpH1* in R3 region and for the allele $ph4^{R149}$ characterized by the presence of the transposon *Thp6* in R2 region of the coding sequence.

To perform functional analyses in *an2* background, two different mutant lines were used, W115 and W59. The *an2* alleles of *Petunia hybrida* line W59 is characterized by a 4-bp insertion after codon 127, just downstream of the conserved MYB domain, resulting in a reading frame shift and a premature stop codon. In the *an2* alleles in the line W115, a 1-bp deletion in codon 127 prevents translation of the C-terminal half of PhAN2 (Quattrocchio *et al.*, 1999).

Table 1. Petunia lines and genotypes used in the experiments of heterologous expression.

LINE	GENOTYPE
I11	*AN1, AN2, AN4, AN9, AN11, HT1, hf1,* $ph4^{V64/R149}$*, fl*
W115	*AN1, an2, an4, AN9, AN11, HT1, hf1, PH4, FL*
W59	*AN1, an2, an4, AN9, AN11, HT1, HF1, PH4, fl*

Plasmid constructs and genetic transformation

The cDNA sequences of *VvMYB5a* and *VvMYB5b* used for complementation analyses were previously isolated by PCR from a grape berry cDNA library.

For heterologous expression of *VvMYBA1,* nested PCRs were performed. The 750 bp ORF was amplified by a first PCR from grapevine (cv Corvina) cDNA (synthesized from RNA isolated from the berry skin at the veraison) using *Pfu* DNA polymerase (Promega) and the primers MYBA1 UTR5 FOR 5'-TTAGAGAAGGAGCCGGTCTC-3' and MYBA1 UTR3 REV 5'-AACATTAAGATTGAAATGGTCT-3'. A second cycle of amplification was conducted with the primers MYBA1 COMPL FOR 5'-ATGGAGAGCTTAGGAGTTAGAAA-3' and MYBA1 COMPL REV 5'-TACTTGTGTGTTGGGTGTAT-3'.

The generated PCR fragments were purified and directionally cloned into the Gateway entry vector pENTR/D-TOPO (Invitrogen) thanks to the 5'-CACC sequence in the forward primer. After the sequencing, the coding sequences were transferred into the binary overexpression vector pK7GW2,0 (Laboratory of Plant Systems Biology, PSB; Ghent University, Belgium) by site-specific recombination. The constructs were then inserted into *Agrobacterium tumefaciens* EHA105 by electroporation and the transformed strains were then used for petunia transformation.

The genetic transformation of *Petunia hybrida* was obtained with leaf disk infiltration by *Agrobacterium tumefaciens* as previously described by van der Meer et al. (1999). Regenerated transgenic shoots were deflasked into soil and hardened off in a temperature controlled glasshouse.

Transcriptomic analyses

For gene expression analyses performed in transgenic plants, total RNA from petunia flowers was isolated using in the TRIzol® Reagent (Invitrogen) and following the manufacturer's instructions.

For the expression profile, 1 µg of total RNA was first treated with *DNase I* (Promega) and then reverse transcribed using the *Improm-II*^{TM} *_Reverse Transcriptase* (Promega) according to the manufacturer's instructions. The transcriptional profile was analyzed by real time RT-PCR as described by Zenoni *et al*. (2010), using the SYBR Green PCR master mix (Applied Biosystems) and a Mx3000P real time PCR system (Stratagene).

To estimate the transcript amounts of *VvMYBA1*, *VvMYB5a* and *VvMYB5b*, gene-specific primers designed were MYBA RT FOR and MYBA RT REV, MYB5a FOR and MYB5a REV, MYB5b FOR and MYB5b REV, respectively (Table 2).

The expression of anthocyanin structural genes *PhCHSa, PhF3H, PhDFRa* and *PhAN9* and pH structural genes *PhPH5* and *PhCAC 16.5* was evaluated using the combination of primers reported in table 2.

Table 2. List of primer used for expression analyses by Real time RT-PCR.

GENE	ACCESSION NUMBER	PRIMER NAME	SEQUENCE 5'-3'
VvMYBA1	BAD18980	MYBA RT FOR	TCACCATGGAGAGCTTAG
		MYBA RT REV	AAGGAAAGTGGCATCTGG
VvMYB5a	AAS68190	MYB5a FOR	TGTGAAGAGAGAAGGTGAAG
		MYB5a REV	ATGGAGGCGAAGAATGAGAT
VvMYB5b	AAX51291	MYB5b FOR	CTCCATCCTTCATCTTCTTCA
		MYB5b REV	GCCTTCTCCTTCTTTCTTGA
PhAN9	Y07721	AN9 FOR	GGTCTGCCTGATAGAGTT
		AN9 REV	TACAGCCATTTGGACAAG
PhAN1	AF260918	AN1 EXON 6 FOR	TCCACCATCCGCTGGCACTA
		AN1 EXON 6 REV	CCAGCTAATGGAAGTGTCAG
PhCAC 16.5	AY371317	CAC 16.5 FOR	GAGATGGACCAACATCTG
		CAC 16.5 REV	GTCAAATGGTTCCAGGTA
PhCHSa	X14591	CHS FOR	GTGACAGTGGAGGAGTTAT
		CHS REV	GTTCAGCCACACCTACAA
PhDFRa	X79723	DFR FOR	AAGCGGACTTGACAGTAG
		DFR REV	CCTATGGATTTCGAGTCC
PhF3H	AF022142	F3H FOR	AGCCAATCTACAGGGTGAA
		F3H REV	TGTCTGGCCATCTAGAGT
PhPH5	DQ888719	PH5 FOR	GCCAACAGTCCTATCAGT
		PH5 REV	GAAGAGATGGCTGGTATG

All quantifications were normalized to actin cDNA fragments amplified in the same conditions by primers ACT1 5’-ATCCCAGTTGCTGACAATAC-3’ and ACT2 5’-GGCCCGCCATACTGGTGTGAT-3’. Each expression value was determined in triplicate. The PCR involved a 50°C hold for 2 min and a 95°C hold for 10 min followed by 40 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 20 s. Nonspecific PCR products were identified by the dissociation curves. Amplification efficiency was calculated from raw data using LingRegPCR software (Ramakers *et al.*, 2003). The relative expression ratio value was calculated for development time points relative to the first sampling time point (post fruit set) according to the Pfaffl equation (Pfaffl, 2001). SE values were calculated according to Pfaffl et al. (2002).

pH assay

The pH of petal extracts was measured by grinding the petal limb of the corolla in 6 mL of distilled water. The pH was measured directly (within 1 min) with a normal pH electrode to avoid the possibility that atmospheric CO^2 would alter the pH of the extract. Each pH value is the mean of ten measures. The actual pH values measured for specific plants showed some variation in time, possibly as a result of variable environmental conditions in the greenhouse, but the differences between distinct genotypes were constant. Therefore, the absolute pH values can be reliably compared between samples/genotypes that were measured within one experiment.

HPLC analyses and quantification of anthocyanins

To measure anthocyanin content, 200 mg of ground petal limb tissue was extracted in a solution 99% methanol - 1% HCl. After sonicatication and centrifugation, each sample was analyzed by spectophotomer in the range from 400 to 600 nm.

For HPLC analysis, powdered samples were extracted with three volumes of ice-cold methanol containing 0.1% formic acid. A HPLC system (Beckman Coulter System Gold 127, Solvent Module) with a Rheodyne sample injector, was used. The system was fitted with a 150- x 2.1-mm Alltima HP C183µm column and a 7.5x 2.1-mm guard column (Alltech Associates). Two solvents were used for separation: 5% (v/v) formic acid / 5% (v/v) acetonitrile in water (solvent A) and 100% acetonitrile (solvent B). After injecting 20 µl of sample at flow rate of 200 µl / min, a solvent gradient was established from 0 % to 10 % B in 5 min, from 10 % to 20 % B in 20 min, from 20 % to 25 % B in 5 min and from 25 % to 70 % B in 15 min. A 20 min equilibration followed each analysis, which was carried out in duplicate. Chromatography data were extracted using metAlign software (http://metAlign.nl; RIKILT Institute of Food Safety).

Microarray chips construction, hybridization and data analysis

The oligonucleotide probes were designed using the Oligo Array 2.1 software (Rouillard *et al.*, 2003), and Custom 90K CombiMatrix arrays were prepared as described by Bellin et al. (2009). One microgram of the total RNA from petals of transgenic petunias *an2 35S::VvMYBA1*, *35S::VvMYB5a* and *35S::VvMYB5b* and mutant line *an2*, was used for each array hybridization. RNA labeling, hybridization reaction, data extraction and quantile data normalization were performed as described in Bellin et al. (2009). Pearson correlation of three biological replicates for each sample ranged from 0.96 to 0.99. A gene was considered expressed when the probe signal was higher than threshold, calculated as the 97th percentile of the intensities from the negative controls, in at least two replicates. Differentially expressed genes were defined by multiclass comparison method of Significance Analysis of Microarray (Tusher *et al.*, 2001), with FDR = 0,2%.

RESULTS

‘Corvina’ VvMYB5a and VvMYB5b versus known *Vitis* spp. sequences

The full length coding regions of *VvMYB5a* and *VvMYB5b* were previously amplified from grapevine ‘Corvina’ cDNA library. *VvMYBA1* sequence was isolated from berry skin cDNA at veraison to be used as control in the heterologous experiments.

The analysis of the isolated sequences revealed that the predicted VvMYB5b protein is identical to the sequence from ‘Cabernet Sauvignon’; for VvMYB5a, the sequences isolated from the two cultivars share 98% similarity. We observed that one of the five different amino acids is located in one of the motifs at the C-terminus that are conserved in all MYB factors of “G20” cluster (Quattrocchio *et al.*, 2006). Regarding VvMYBA1, the alignment showed 99% similarity between ‘Corvina’ and ‘Cabernet Sauvignon’, respectively.

Since the last release of the gene prediction V1 and automatic annotation of the 12X sequence assembly of the grape genome, it has been also possible to blast the ‘Corvina’ sequences against the ‘Pinot noir’ genome. In this case the amino acid similarity is 98% for VvMYB5b and 99% for both VvMYBA1 and VvMYB5a, respectively (Figure 1). When compared to the sequences of ‘Pinot noir’ and ‘Cabernet Sauvignon’, we observed that VvMYBA1 protein sequence isolated from ‘Corvina’ presents one different amino acid at position 12 in the R2 region.

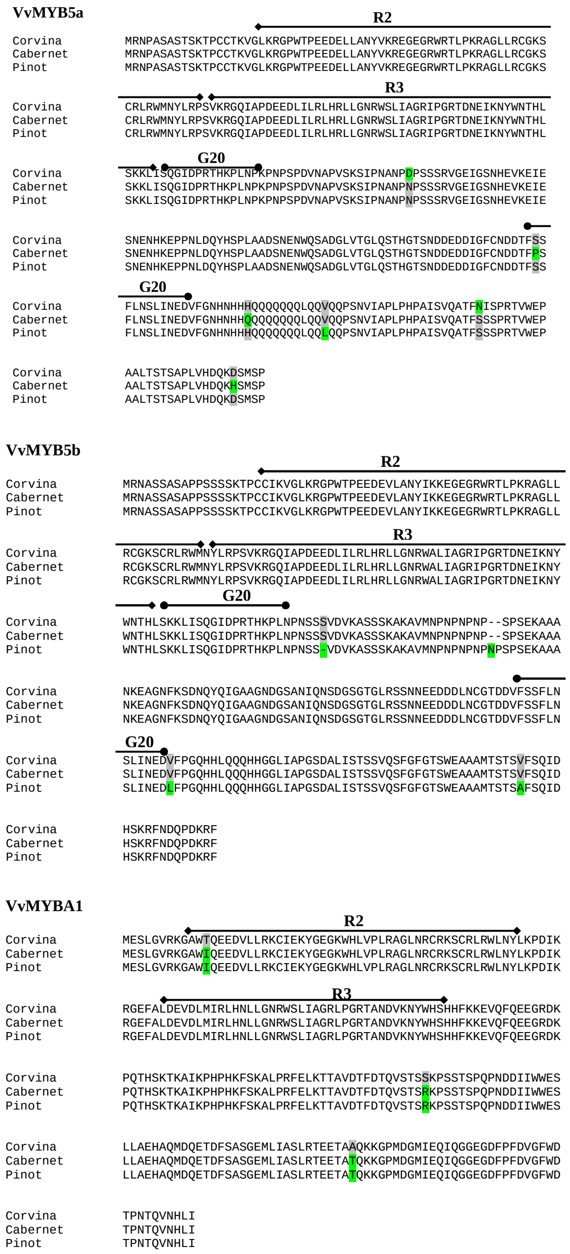

Figure 1. Alignment of VvMYB5a, VvMYB5b and VvMYBA1 predicted amino acidic sequences from 'Corvina', 'Cabernet Sauvignon' and 'Pinot Noir' cultivars. Different amino acids are indicated in green. The R2 and R3 repeats that make up the MYB domain are indicated above the alignment. In VvMYB5a and VvMYB5b sequences, two conserved domains called 'G20'are indicated above the alignment.

Characterization of petunia *ph4* mutant line expressing *VvMYBa* and *VvMYB5b*

To analyze whether the high sequence similarity to PhPH4 may reflect a similar function and to provide more information about their biological roles, *VvMYB5a* and *VvMYB5b* were expressed independently in a petunia *ph4* mutant line. The *ph4* mutant used for this study is a hybrid between the lines R159 e V64 harbouring two unstable *ph4* alleles (Quattrocchio *et al.*, 2006). The choise of this hybrid is dictated by the fact that most inbred petunia lines are untransformable, while hybrids can be easily transformed. The mutation in *PH4* locus causes the loss of the capacity to activate the vacuolar acidification in the epidermal cells of the petal, resulting in a shift to a more bluish flower. Moreover, the *ph4* mutation triggers also the complete fading of flower colour and the disappearance of anthocyanins after opening of the flower bud, if combined with a dominant allele at the FADING locus (Wiering, 1974; de Vlaming, 1982).

Both *35S::VvMYB5a* and *35S::VvMYB5b* constructs were able to complement the *ph4* mutation, restoring both the vacuolar pH and the red-purple flower colour. However, we obtained a range of phenotypic effects, from the fully complementated evenly red-pigmented nonfading corollas to a blue-violet fading corollas similar to the mutant *ph4* flowers. We selected three transgenic plants for each construct and verified that the different levels of phenotypic complementation were correlated to the transgene expression (data not shown). For a deeper characterization, we selected the transgenic line with highest expression of the transgene for each construct (Figure 2A). They produced a reddish flower with a lower pH of the crude petal extract as compared to the untransformed line (Figure 2A).

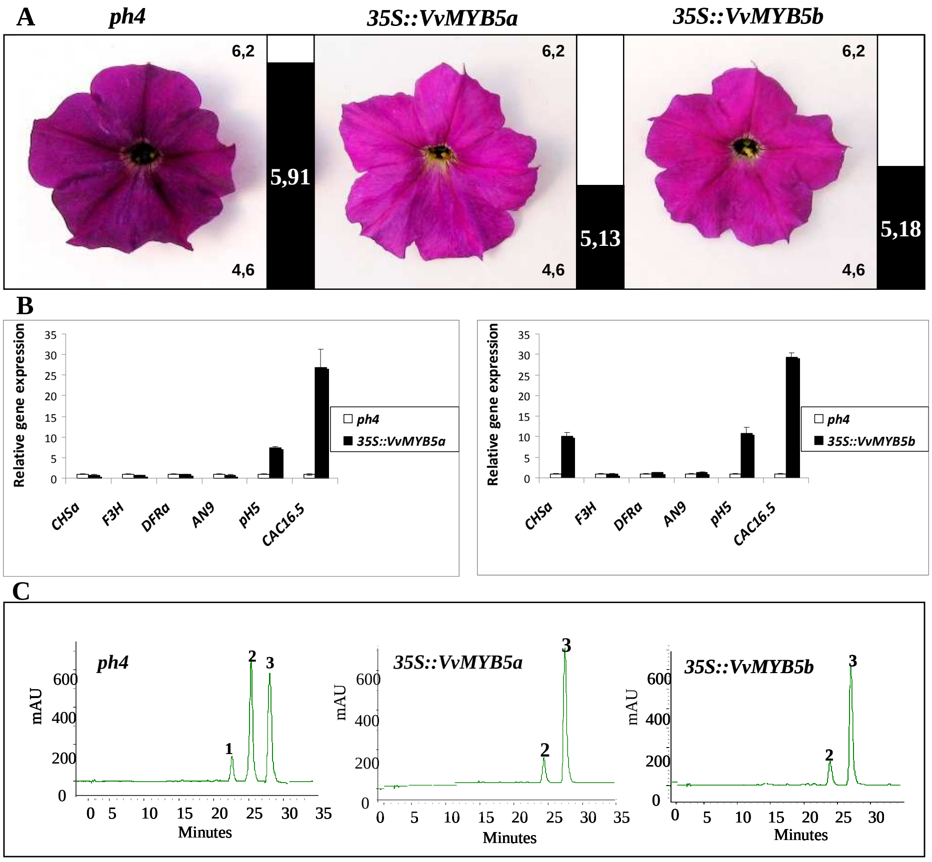

Figure 2. Complementation study of the *VvMYB5a* and *VvMYB5b* in petunia *ph4* mutant line.

A) Fully complementation of *ph4* mutation by *35S::VvMYB5a* and *35S::VvMYB5b* in comparison to the untransformed plant. Bars on the right site of the pictures show the pH of the crude petal homogenates.

B) Expression analyses of structural genes for anthocyanin synthesis and vacuolar acidification in flowers of transgenic plants and *ph4* mutant line by real time RT-PCR. Abbreviations are as follows: CHSa, chalcone synthase a; F3H, flavanone 3-hydroxylase; DFRa, dihydroflavonol reductase a; CAC 16.5, cysteine proteinase.

C) HPLC analyses of methanol-extractable anthocyanins in petals of stage 6 flower buds from lines *ph4 35S::VvMYB5a*, *ph4 35S::VvMYB5b* and the control *ph4*. Peaks 1, 2 and 3 are referred to Delphinidin 3-(p-coumaroyl)-rutinoside-5-glucoside, Petunidin 3-(p-coumaroyl)-rutinoside-5-glucoside, Malvidin 3-(p-coumaroyl)-rutinoside-5-glucoside, respectively.

Expression analyses performed on RNA extracted from petals of *35S::VvMYB5a* and *35S::VvMYB5b* plants indicated that, like PhPH4, both the regulators were able to activate the expression of structural genes associated with the vacuolar acidification pathway (Quattrocchio *et al.*, 2006). In detail, the expression of the P-ATPase proton pump *PhPH5* and the putative cysteine proteinase *PhCAC 16.5* genes was higher than in flowers of the mutant line (Figure 2B), resulting in a lower pH of the lumen.

Spectroscopic analyses of petal extracts (540 nm) did not reveal any increase in the anthocyanin content in the transgenic plants in comparison to the untransformant *ph4* line (data not shown). In fact, the expression of the anthocyanin structural genes, *PhCHSa, PhF3H, PhDFRa* and *PhAN9* did not appear to be induced in the transgenic plants in comparison to the *ph4* mutant as shown in figure 2B.

To test whether alterations in pigment synthesis also contributed to the color change in petals, we analyzed anthocyanin content in *35S::VvMYB5a*, *35S::VvMYB5b* and *ph4* petals by HPLC. Figure 2C shows that both transgenic plants accumulate more malvidin 3-(p-coumaroyl)-rutinoside-5-glucoside, while the amount of delphinidin 3-(p-coumaroyl)-rutinoside-5-glucoside and petunidin 3-(p-coumaroyl)-rutinoside-5-glucoside decreases. It is possible that VvMYB5a and VvMYB5b regulate genes involved in the modification of the anthocyanidins, such as *3'AMT* and *3'5'AMT*, that were not considered in the real time RT-PCRs because the sequences were not available. Only recently, three anthocyanin methyltransferase genes *MT*, *MF1* and *MF2* from *Petunia hybrida* have been isolated and characterized, but these data are still unpublished (Provenzano, 2011).

Besides the principal role in the regulation of the anthocyanin pathway, the petunia MYB transcription factor AN2 is also partially involved in the vacuolar acidification (Verweij *et al.*, 2008). To test if also the grape homolog VvMYBA1 could participate to this regulatory network, the *ph4* mutant line was transformed with the construct *35S::VvMYBA1*. However, none of the transgenic plants displayed any rescue of the *ph4* phenotype. Real time RT-PCR confirmed in fact that VvMYBA1 is not able to activate the pH structural genes and to induce vacuolar acidification in petunia (data not shown).

Taken together, this results indicated that *VvMYB5a* and *VvMYB5b* are functional homologs to *PhPH4*. Both genes regulate the expression of pH structural genes in petunia, restoring the *PH4* phenotype.

Heterologous expression of *VvMYB5a, VvMYB5b* and *VvMYBA1* in *an2* mutant lines

Indications about the ability of VvMYB5a and VvMYB5b to regulate the anthocyanin biosynthetic pathway was previously provided by experiments in tobacco plants (Deluc *et al.*, 2006; Deluc *et al.*, 2008). To better explore the putative role in flavonoid biosynthesis, the coding sequences of *VvMYB5a* and *VvMYB5b* were constitutively expressed in the petunia *an2* line W115. This stable mutant is characterized by 1-bp deletion in the *AN2* locus causing translation of a truncated protein and a partial inactivation of anthocyanin synthesis in epidermal cells of petals. As results, the loss of pigmentation is the main phenotype observed in *an2* flowers, although a moderate increase of the vacuolar pH is also recorded (Quattrocchio *et al.*, 1999).

The coding sequence of *VvMYBA1*, the grape closest homolog to PhAN2, was also expressed in *an2* mutant line, in order to compare and emphasize differences in regulative mechanisms between the MYB TFs. We obtained 3, 5 and 4 PCR positive plants for *35S::VvMYB5a*, *35S::VvMYB5b* and *35S::VvMYBA1* expression, respectively. Among all of them, we selected the transformant with highest expression of the each transgene.

All constructs caused an increase of pigmentation even if with different intensity. In detail, flowers of *VvMYB5a* and *VvMYB5b* plants exhibited pale pink pigmentation mainly in the veins of the petals (Figure 3A), more evident in *VvMYB5b* plants. On the other side, the plants expressing *VvMYBA1* were characterized by stronger, slightly more bluish pigmentation distributed homogenously in the adaxial epidermis of the petals. Only *VvMYBA1* plants presented anthocyanin accumulation also in the epidermis of leaves (Figure 3B).

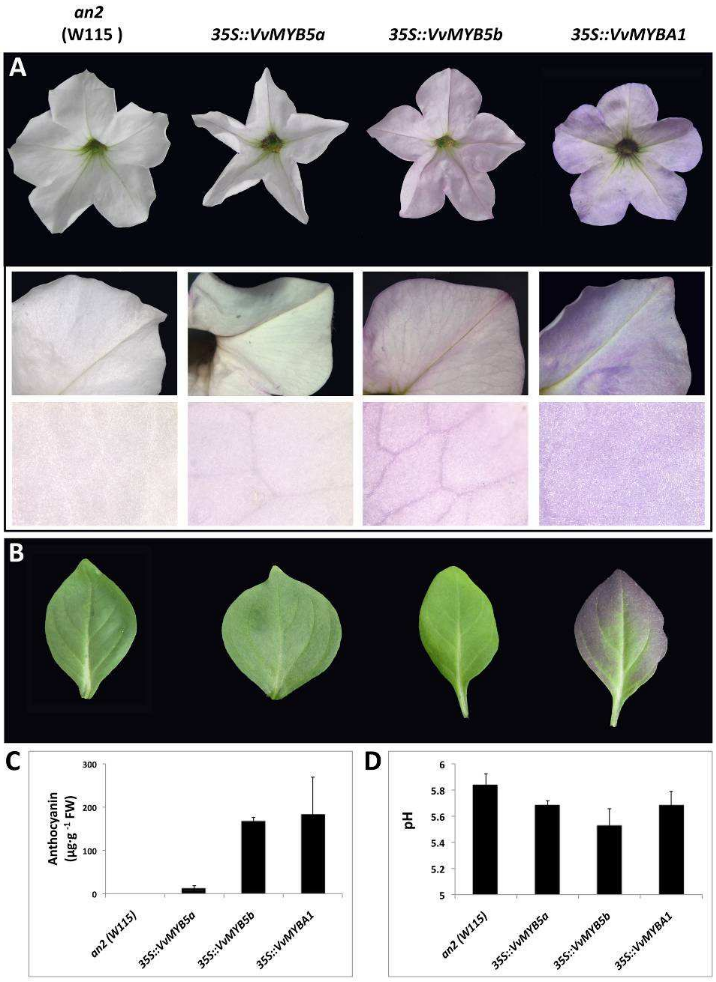

Figure 3. Complementation study of the *VvMYB5a*, *VvMYB5b* and *VvMYBA1* in petunia *an2* mutant line W115.

A) Phenotype of *35S::VvMYB5a*, *35S::VvMYB5b* and *35S::VvMYBA1* plants in comparison to the untransformed plant. Higher magnifications of petal sections reveal the pigment localization in the veins for *35S::VvMYB5a* and *35S::VvMYB5b* flowers and in the epidermis for *VvMYBA1* flowers.

B) Leaves of *35S::VvMYB5a*, *35S::VvMYB5b* and *35S::VvMYBA1* plants in comparison to the untransformed line W115. The expression of *VvMYBA1* induced anthocyanin accumulation in leaf epidermis.

C) Total anthocyanin content (µg/g FW) of petal extracts from control and transgenic plants determined by spectrophotometer at 540 nm. Purified malvidin 3,5-diglucoside standard was used as a control. Data are the mean of three replicates +/- SD.

D) pH values of petal limb homogenates of the control and transgenic plants. Each pH value is the mean of ten replicates +/- SD.

Quantification of anthocyanin content of petal extracts by spectrophotometry (540 nm) confirmed the phenotypes of the transgenic plants (Figure 3C). In fact, *35S::VvMYB5b* and *35S::VvMYBA1* petals accumulate more anthocyanins (166.9 µg/g FW and 182.6 µg/g FW, respectively) when compared to *35S::VvMYB5a* (12.06 µg/g FW) and *an2* mutant line (undetectable). HPLC analyses of the transgenic plants revealed also different pigmentation profiles. *VvMYB5a* and *VvMYB5b* plants produced mainly delphinidin 3-rutinoside (Figure 4B and C), a di-substituted anthocyanin that in petunia is acylated and methylated to originate petunidin and malvidin. VvMYBA1 instead is able to induce other modifications downstream the pathway in the *an2* mutant which consequently accumulates petunidin 3-(p-coumaroyl)-rutinoside-5-glucoside as main anthocyanin (Figure 4D). Among the polyphenols, feruloyl-malic acid and caffeoyl-malic acid residues were detected only in petal extracts of *35S::VvMYB5b* flowers as shown in figure 4C. The expression of *VvMYB5a* and *VvMYB5b* causes also an increase of the flavonols myceritin malonyl-diglucosyde and miceritin diglucoside (data not shown).

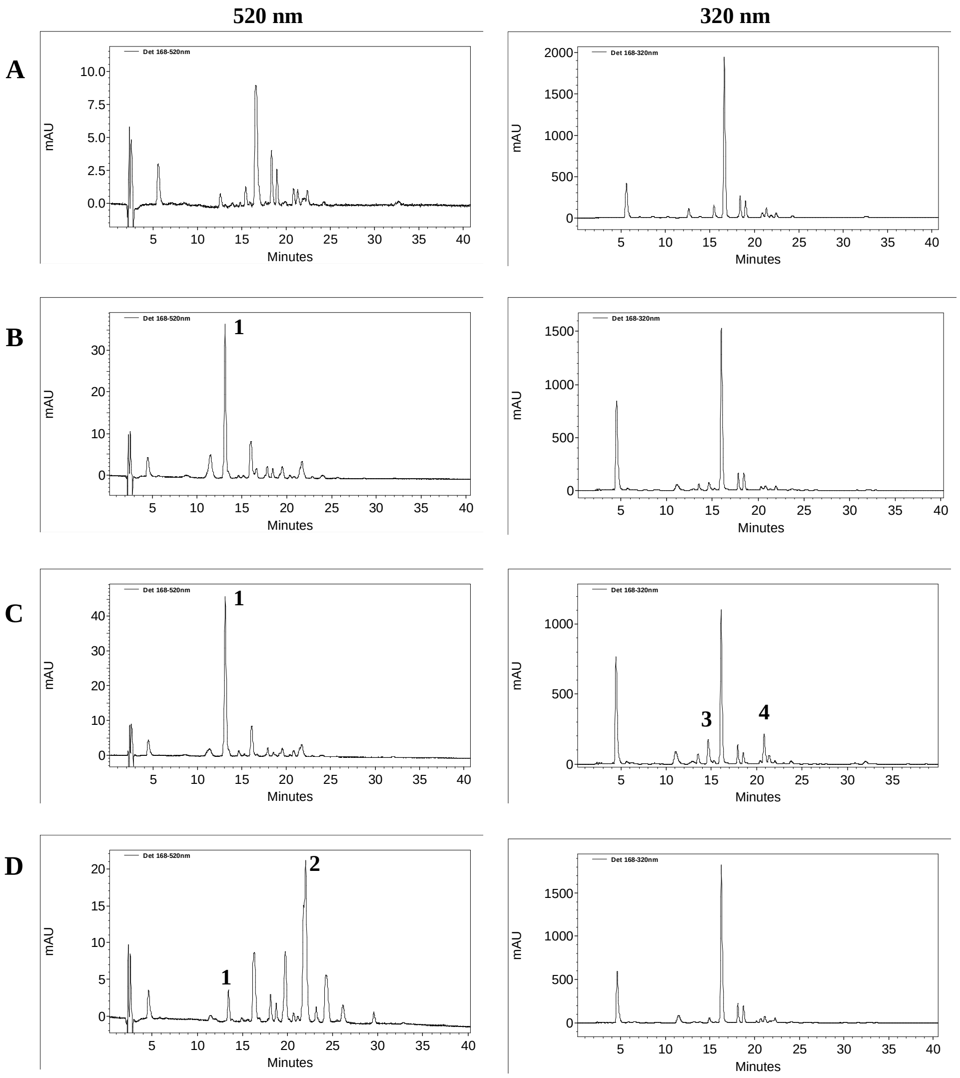

Figure 4. HPLC analysis of methanol-extractable anthocyanins (520 nm) and polyphenols (320 nm) in petals of flower buds from *an2* mutant line (**A**) in comparison to the transgenic plants *35S::VvMYB5a* (**B**), *35S::VvMYB5b* (**C**) and *35S::VvMYBA1* (**D**).

Reaction products were identified according to their absorption spectra and retention time. The data shown are representative of two biological replicates. The identification numbers correspond to delphinidin 3-rutinoside (1), petunidin 3-(p-coumaroyl)-rutinoside-5-glucoside (2), caffeoyl-malic acid (3) and feruloyl-malic acid (4).

Expression analysis of anthocyanin structural genes revealed that *PhDFRa* gene appeared up-regulated in all the transgenic lines, while *PhAN9* expression was induced only by VvMYBA1 and, less, by VvMYB5b (Figure 5). By contrast, the expression of the early structural genes considered (*PhCHSa* and *PhF3H*) was not modified, suggesting that all these MYB factors are able to regulate only the late genes of the flavonoid pathway. Regarding the differences of colour observed, we hypothezed a different ability of these MYB transcription factors to regulate vacuolar acidification. The pink coloration of the *VvMYB5a* and *VvMYB5b* petals is associated with a lower vacuolar pH, although the pH value was higher in *VvMYB5a* than in *VvMYB5b* petals. On the other side, *VvMYBA1* bluish-violet flowers presented pH similar to the *an2* mutant line, indicating that, differently from VvMYB5a and VvMYB5b, VvMYBA1 can regulate only the anthocyanin synthesis. This aspect distinguishes VvMYBA1 also from the petunia homolog AN2, which is also partially involved in the control of the vacuolar pH (Verweij *et al.*, 2008). These data are confirmed by expression analysis of the pH structural genes *PhCAC 16.5* and *PhPH5,* induced slightly by VvMYB5a and VvMYB5b and not by VvMYBA1 (Figure 5).

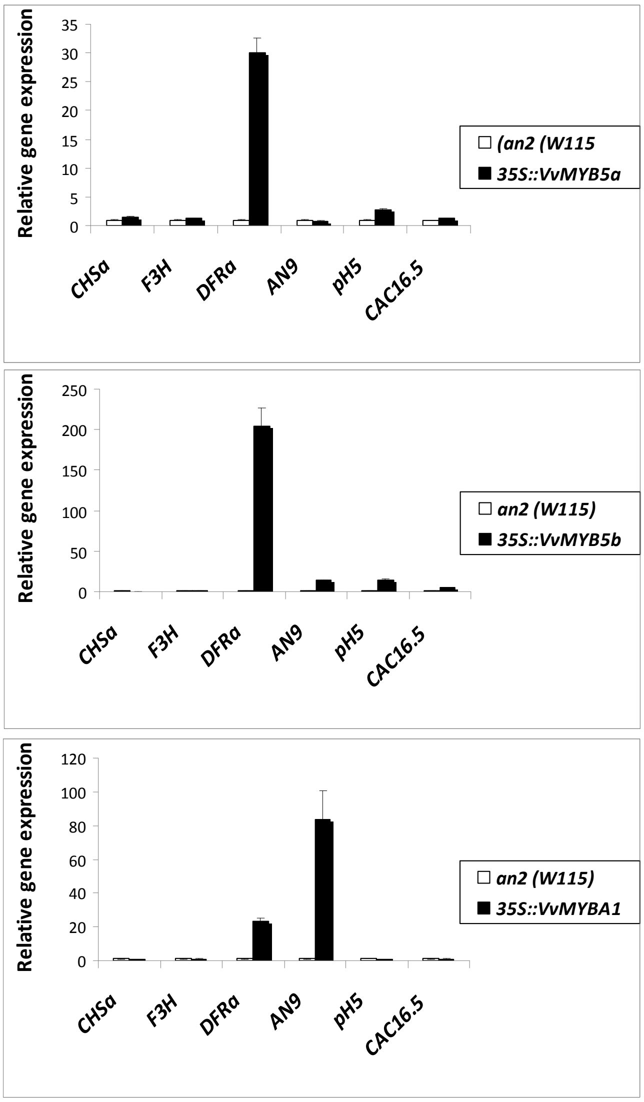

Figure 5. Expression analyses of structural genes for anthocyanin synthesis and vacuolar acidification in flowers of transgenic plants and *ph4* mutant line by real time RT-PCR. Abbreviations are as follows: CHSa, chalcone synthase; F3H, flavanone 3-hydroxylase; DFRa, dihydroflavonol reductase; CAC 16.5, cysteine proteinase.

Other phenotypic differences were observed among *VvMYB5a*, *VvMYB5b* and *VvMYBA1* plants. In particular, *VvMYBA1* expressing plants presented anthocyanin accumulation in the adaxial epidermis of leaves.

As the bHLH factor PhAN1 is required for anthocyanin production in petunia tissues and it is not expressed in leaves, we hypothesized that *VvMYBA1* expression could trigger the expression of *PhAN1* in leaves. Real time RT-PCR performed on cDNA of leaf tissue of the transgenic plants revealed that VvMYBA1 can strongly induce *PhAN1* expression, explaining the increased pigmentation in the leaves (Figure 6). The same effect was previously achieved by ectopic expression of *PhAN2*, causing induction of *PhAN1* expression in leaf tissue and pigmentation in the veins (Quattrocchio *et al.*, 1998). The same analysis was performed on *VvMYB5a* and *VvMYB5b* transformed leaves, revealing that only *VvMYB5b* could induce *PhAN1* expression even if at lesser extent than *VvMYBA1*.

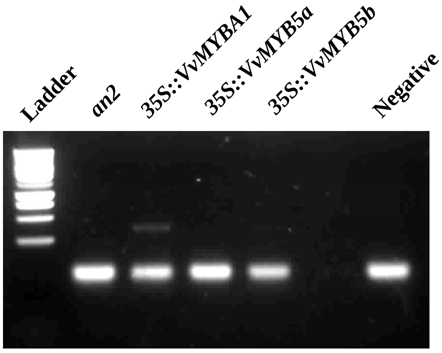

Figure 6. *PhAN1* expression by real time RT-PCR. The analysis was conducted on petal cDNA of the mutant *an2*, transgenic plants transformed with *35S::VvMYBA1, 35S::VvMYB5a* and *35S::VvMYB5b* constructs.

Different dimensions of leaves and flowers were observed for *VvMYB5a* expressing plants (Figure 3A and B). To verify whether the increased size of the petal limbs correlates with differences in the size of epidermal cells, we measured the cell surface in petal adaxial and abaxial epidermis of *an2* untransformed line and transgenic lines (Figure 7). A *t*-test (using P values <0.001) revealed that the cells of the abaxial epidermis of *35S::VvMYB5a* petals are larger than the *an2* cells.

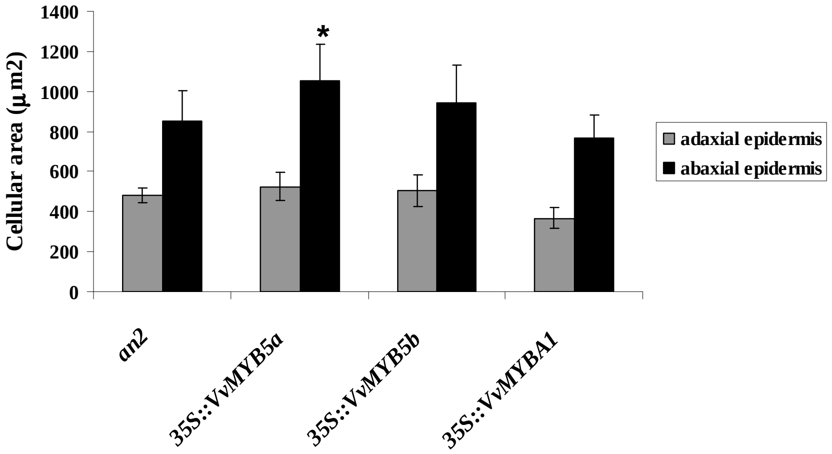

Figure 7. Measurements of cell surface of *an2* (W115), *35S::VvMYB5a*, *35S::VvMYB5b* and *35S::VvMYBA1* petals in the respective adaxial and abaxial epidermis. Asterisk indicates significantly different from *an2* mutant line (*t*-test, P <0.001).

The *an2* genotype used for these complementation analyses (W115) is characterized also by the mutation *hf1-1* for flavonoid-3'5'-hydroxylase. As a consequence, the precursors of the flavonoid pathway are preferentially driven to the flavonol production, making less evident the anthocyanin accumulation in the transgenic flowers. To emphasize the pigmentation in the petals, *VvMYB5a*, *VvMYB5b* and *VvMYBA1* plants in *an2* background were crossed with the *an2* mutant line W59, which bears the wild type *HF* allele and is mutated in *FL* locus for the flavonol synthase. In this way, the pathway is pushed to the anthocyanin synthesis.

As a result of the cross, the accumulation of anthocyanins was more evident only in petals of W115xW59 *VvMYBA1* plants (Figure 8A), confirming VvMYBA1 the real ortholog of PhAN2. For W115xW59 *VvMYB5a* and *VvMYB5b* plants, no increase in corolla pigmentation compared to the respective expression in line W115, could be observed (Figure 8B). Results of vacuolar pH analyses (Figure 8C) confirmed VvMYB5a and VvMYB5b as pH regulators. They resulted to be also partially involved in the regulation of anthocyanin biosynthesis, causing a weak pigmentation of petals in comparison to VvMYBA1. Moreover, similarly to the expression in line W115, VvMYB5a induced other changes in plant architecture, such as altered dimensions of leaves and petals, suggesting other roles in different developmental processes.

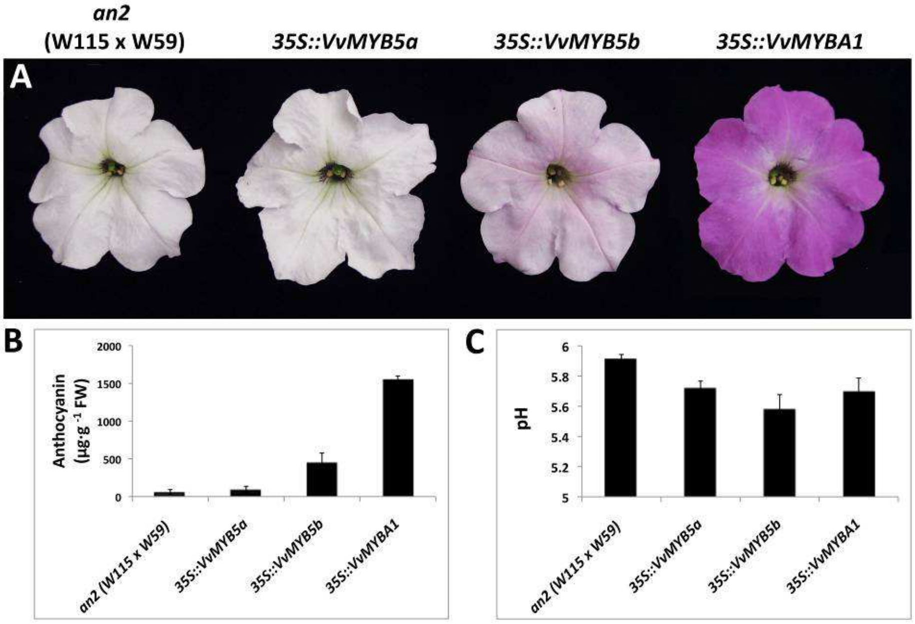

Figure 8. Expression of *VvMYB5a*, *VvMYB5b* and *VvMYBA1* in petunia *an2* mutant line W115xW59.
A) Phenotype of *35S::VvMYB5a*, *35S::VvMYB5b* and *35S::VvMYBA1* flowers in comparison to the untransformed line W115xW59.
B) Total anthocyanin content (µg/g FW) of petal extracts from control and transgenic petal limbs determined by spectrophotometer at 540 nm. Purified malvidin 3'5'-diglucoside standard was used as a control. Data are the mean of three replicates +/- SD.
D) pH values of petal limb homogenates of the control and transgenic plants. Each pH value is the mean of ten replicates +/- SD.

Microarray analysis

In order to investigate which are the target genes and to highlight specific roles for each MYB factor, a microarray analysis was performed using a newly developed chip based on transcriptome of *Petunia axillaris* (Zenoni *et al.*, 2011). Three independent biological replicates of petals were considered for W115 line and for the same line transformed with *35S::VvMYB5a*, *35S::VvMYB5b* and *35S::VvMYBA1* constructs.

Considering a fold change > |2|, 34 oligonucleotides presented an increase in their hybridization signal for *VvMYB5a* (Table 3), 72 for *VvMYB5b* (Supplementary table 1) and 127 in the case of *VvMYBA1* transformants (Supplementary table 2). We have searched for a putative function for all these sequences by referring to the annotation reported by Zenoni *et al.* (2011).

Table 3. The 34 most induced genes in *35S::VvMYB5a* petals compared to the untransformed line *an2*

SEQ_ID	GENE DESCRIPTION	FC
PETAX064323_Contig2_404_40_S	no hit	6.2
PETAX053565_Contig1_240_40_S	predicted protein At1g67920 (*Arabidopsis thaliana*)	5.4
PETAX006031_Contig1_373_40_X2	4-coumarate:coenzyme A ligase (*Capsicum annuum*)	4.8
PETAX002335_Contig1_634_40_S	CXE carboxylesterase (*Malus pumila*)	4.8
PETAX037256_Contig1_400_40_S	Unknown protein (*Vitis vinifera* GSVIVT00023233001)	3.7
PETAX007193_Contig1_375_39_S	Unknown protein (*Nicotiana tabacum* TT-06_I15)	4.1
PETAX020993_Contig1_332_39_S	no hit	3.7
PETAX071872_Contig1_575_39_S	Unknown protein (*Vitis vinifera* GSVIVT00011405001)	3.8
PETAX088705_Contig1_191_39_S	no hit	2.7
PETAX022705_Contig1_452_40_S	Glutaredoxin (*Solanum tuberosum*)	3.7
PETAX064323_Contig1_241_39_X2	no hit	3.6
PETAX051860_Contig1_368_37_S	Malate dehydrogenase (*Solanum tuberosum*)	3.3
PETAX071906_Contig1_246_40_S	Predicted protein (*Populus trichocarpa)*	3.1
PETAX062758_Contig1_314_39_S	Xyloglucan endotransglucosylase-hydrolase XTH3 (*Solanum lycopersicum*)	3.1
PETAX076643_Contig1_551_37_S	Putative uncharacterized protein (*Vitis vinifera*)	3.1
PETAX018551_Contig1_621_39_S	Putative caffeoyl-CoA 3-O-methyltransferase (*Capsicum annuum*)	3.0
PETAX024562_Contig1_393_35_S	no hit	3.0
PETAX010945_Contig1_488_39_S	Putative uncharacterized protein (*Capsicum annuum*)	2.9
PETAX039359_Contig1_657_40_S	Phosphorylase (*Ricinus communis*)	2.9
PETAX060284_Contig1_464_40_S	6-phosphogluconate dehydrogenase (*Ricinus communis*)	2.8
PETAX026374_Contig1_274_38_S	no hit	2.8
PETAX054602_Contig1_70_40_S	Hf2 gene for flavonoid 3'. 5'-hydroxylase (*Petunia integrifolia* var. integrifolia)	2.8
PETAX001318_Contig2_637_37_S	no hit	2.5
PETAX003654_Contig1_605_40_S	Putative nodulin-like protein (*Gossypioides kirkii*)	2.5
PETAX083272_Contig1_278_40_S	Unknown protein *(Vitis vinifera* GSVIVT00033459001)	2.5
PETAX097399_Contig1_658_40_S	Putative shikimate kinase (*Fagus sylvatica*)	2.5
PETAX027137_Contig1_482_40_S	Unknown protein (*Vitis vinifera* GSVIVT00033838001)	2.4
PETAX040559_Contig1_272_40_S	Predicted protein (*Populus trichocarpa*)	2.4
PETAX044933_Contig4_395_37_S	Actin depolymerizing factor (*Nicotiana benthamiana*)	2.3
PETAX015624_Contig1_358_38_S	no hit	2.2
PETAX014081_Contig2_457_40_S	Lipid-transfer protein (*Nicotiana attenuata*)	2.2
PETAX018009_Contig3_264_35_S	Putative acyl-CoA synthetase (*Capsicum annuum*)	2.2
PETAX036904_Contig1_411_40_S	no hit	2.0
PETAX017355_Contig1_377_40_S	Unknown protein (*Solanum lycopersicum* clone BAC C09HBa0142I14)	2.0

Among the genes induced by *VvMYB5a* expression (Table 3), we identified shikimate kinase linked to the metabolism of aromatic amino acids, precursors of the flavonoid pathway. Three genes are related to the general phenylpropanoid pathway (4-coumarate:coenzyme A ligase, caffeoyl-CoA 3-O-methyltransferase and a putative acyl-CoA synthetase). In contrast with the results obtained by real time RT-PCR

(Figure 5), no induction of *DFR* was revealed in *35S::VvMYB5a* samples and the only member of the flavonoid pathway corresponds to *HF2* gene for flavonoid 3',5'-hydroxylase. This transcript was also found up-regulated in *VvMYB5b* petals. The induction of *HF2* is in line with accumulation of myceritin and delphidin-derivates revealed in petals of both transgenic plants. The expression of *VvMYB5b* led to the induction of many other genes related to the flavonoid pathway (Table 4 and Supplementary table 2) as *DIF-F* , encoding a cytochrome B5 (de Vetten *et al.*, 1999), anthocyanidin synthase and two genes for dihydroflavonol-4-reductase.

Table 4. The 30 most induced genes in *35S::VvMYB5b* petals compared to the untransformed line *an2*

SEQ_ID	**GENE DESCRIPTION**	**FC**
PETAX006060_Contig1_540_40_S	no hit	33.8
PETAX078379_Contig1_297_40_S	RNase Phy4 *(Petunia hybrida*)	24.7
PETAX060521_Contig1_487_37_S	Serine carboxypeptidase *(Ricinus communis)*	12.2
PETAX031168_Contig1_435_40_S	Endochitinase *(Capsicum annuum)*	11.0
PETAX046579_Contig1_286_40_S	3'-N-debenzoyl-2'-deoxytaxol N-benzoyltransferase *(Ricinus communis)*	8.8
PETAX071872_Contig1_575_39_S	Unknown protein (*Vitis vinifera* GSVIVT00011405001)	8.5
PETAX014081_Contig2_457_40_S	Lipid-transfer protein *(Nicotiana attenuata)*	8.5
PETAX062423_Contig1_206_35_S	Dihydroflavonol-4-reductase *(Petunia hybrida)*	6.7
PETAX089413_Contig1_294_40_S	Zinc binding dehydrogenase *(Ricinus communis)*	5.8
PETAX090363_Contig1_305_40_S	Dihydroflavonol-4-reductase *(Petunia hybrida)*	5.7
PETAX068373_Contig1_359_38_S	Polyphosphoinositide binding protein *(Arabidopsis thaliana)*	5.5
PETAX066446_Contig1_373_40_S	Cytochrome B5 *(Petunia hybrida)*	5.4
PETAX088705_Contig1_191_39_S	no hit	5.1
PETAX069683_Contig1_325_36_S	Unknown protein (*Vitis vinifera* GSVIVT00035108001)	5.0
PETAX037256_Contig1_400_40_S	no hit	4.6
PETAX014375_Contig2_266_40_X2	no hit	4.5
PETAX054602_Contig1_70_40_S	Hf2 gene for flavonoid 3'. 5'-hydroxylase *(Petunia integrifolia* var. integrifolia)	4.3
PETAX077682_Contig1_200_40_S	Acyltransferase *(Capsicum frutescens)*	4.2
PETAX027410_Contig1_340_39_S	Unknown protein *(Vitis vinifera* GSVIVT00003016001)	4.2
PETAX014375_Contig1_504_39_X2	no hit	4.1
PETAX001393_Contig2_69_40_S	Sucrose synthase 4 *(Solanum tuberosum)*	3.9
PETAX006361_Contig2_12_40_S	Unknown protein *(Nicotiana tabacum)*	3.8
PETAX015426_Contig1_418_40_S	Unknown protein (*Vitis vinifera* GSVIVT00030722001)	3.8
PETAX012531_Contig1_485_40_S	Predicted protein *(Populus trichocarpa)*	3.7
PETAX043185_Contig2_206_38_S	Predicted protein *(Populus trichocarpa)*	3.6
PETAX068472_Contig1_430_39_S	Autoinhibited calcium ATPase *(Populus trichocarpa)*	3.5
PETAX005440_Contig1_256_40_S	Purine transporter *(Ricinus communis)*	3.5
PETAX062686_Contig1_153_35_X2	no hit	3.5
PETAX006470_Contig1_572_35_S	Anthocyanidin synthase *(Ipomoea purpurea)*	3.4
PETAX090339_Contig1_257_40_S	Mtn21-like protein *(Populus trichocarpa)*	3.4
PETAX039849_Contig1_415_37_S	no hit	3.2
PETAX087824_Contig3_164_35_X4	no hit	3.2
PETAX071176_Contig1_459_39_S	CAPRICE *(Gossypium herbaceum)*	3.2
PETAX028060_Contig1_248_40_S	no hit	3.1

We observed also the induction of an ATP-driven Ca^{2+} pump which may play an important role in maintaining low vacuolar pH. However, none of the pH structural genes already shown to be regulated by VvMYB5a and VvMYB5b appeared induced in this experiment. In detail, PhPH3 and PhCAC16.5 did not result to be up-regulated, while *PH5* gene was not represented on the microarrays (Zenoni *et al.*, 2011). In addition to the flavonoid genes, the homolog to the Arabidopsis *CAPRICE (CPC)* was identified. CPC is a MYB factor that may play a negative role in the control of the processes regulated by the complex WD40-MYB-bHLH (Schellmann *et al.*, 2002).

Table 5. The 30 most induced genes in *35S::VvMYBA1* petals compared to the untransformed line *an2*

SEQ_ID	GENE DESCRIPTION	FC
PETAX087824_Contig3_164_35_X4	no hit	65.2
PETAX085370_Contig1_320_39_S	no hit	61.2
PETAX062686_Contig1_153_35_X2	no hit	48.3
PETAX087824_Contig5_224_36_X2	no hit	39.7
PETAX024978_Contig2_286_40_S	no hit	31.0
PETAX066815_Contig2_692_39_S	UDP rhamnose:anthocyanidin-3-glucoside rhamnosyltransferase *(Petunia axillaris)*	30.6
PETAX027976_Contig1_285_40_S	no hit	28.8
PETAX007368_Contig1_256_40_S	Glutathione S-transferase *(Petunia hybrida)*	28.1
PETAX002242_Contig1_360_38_X2	no hit	24.6
PETAX024978_Contig1_381_36_S	no hit	22.2
PETAX025229_Contig1_295_40_X4	Serine carboxypeptidase *(Ricinus communis)*	21.2
PETAX080846_Contig1_125_35_X2	no hit	18.5
PETAX025229_Contig2_28_40_S	Serine carboxypeptidase *(Ricinus communis)*	18.4
PETAX002242_Contig3_255_35_X2	no hit	16.3
PETAX025360_Contig1_392_40_S	no hit	16.0
PETAX100402_Contig1_88_40_S	no hit	15.2
PETAX030315_Contig1_73_36_S	no hit	14.1
PETAX080846_Contig2_13_38_S	no hit	13.6
PETAX060521_Contig1_487_37_S	Serine carboxypeptidase *(Ricinus communis)*	13.2
PETAX091494_Contig1_215_40_S	no hit	13.2
PETAX006874_Contig1_415_40_S	no hit	12.8
PETAX001318_Contig2_637_37_S	no hit	12.2
PETAX080933_Contig1_417_38_S	Anthocyanin 5-O-glucosyltransferase *(Petunia hybrida)*	12.0
PETAX012531_Contig1_485_40_S	Predicted protein *(Populus trichocarpa)*	11.4
PETAX087824_Contig1_144_36_X2	no hit	11.0
PETAX001137_Contig2_206_40_X2	no hit	10.5
PETAX031145_Contig1_439_39_S	Caffeoyl-CoA O-methyltransferase (*Plantago major*)	10.5
PETAX014375_Contig2_266_40_X2	no hit	10.4
PETAX006899_Contig2_29_38_S	no hit	10.4
PETAX066446_Contig1_373_40_S	Cytochrome B5 *(Petunia hybrida)*	9.7
PETAX030315_Contig2_523_40_S	no hit	9.7
PETAX060754_Contig1_309_35_S	no hit	9.0
PETAX024608_Contig1_473_39_S	no hit	8.5
PETAX036148_Contig1_74_40_S	no hit	8.3

Regarding VvMYBA1, the experiment confirmed its role in the regulation of the late anthocyanin structural genes as cytochrome B5 (*DIF-F*), dihydroflavonol-4-reductase and anthocyanidin synthase (Table 5 and Supplementary table 2). Moreover, genes related to anthocyanin glycosilation and acylation as well as genes related to the anthocyanin transport (UDP rhamnose:anthocyanidin-3-glucoside rhamnosyltransferase, anthocyanin 5-O-glucosyltransferase, anthocyanin acyltransferase, anthocyanidin 3-O-glucosyltransferase, glutathione S-transferase and a putative anthocyanin permease) were identified, indicating that, differently from VvMYB5a and VvMYB5b, VvMYBA1 controls also the last steps of the pathway. Moreover, the expression of *VvMYBA1* caused the induction of a caffeoyl-CoA O-methyltransferase, involved in the lignin biosynthesis (Table 5).

A comparison between the results obtained by all microarray analyses (Table 6) revealed that many transcripts remained without any putative function either because of lack of functional information in public data bases (unknown proteins) or because little or no similarity was detected at all with known sequences (no hits). VvMYBA1 and VvMYB5a cause a weak induction of a xyloglucan endotransglucosylase-hydrolase, whose relation with the regulatory functions of the MYB factors is still unclear. In addition to the some flavonoid structural genes above cited, two genes for serine carboxypeptidase, whose function is still unknown, were strongly up-regulated in both *VvMYBA1* and *VvMYB5b* analyses. Among the genes commonly induced by VvMYB5a and VvMYB5b, some lipid-transfer proteins as well as membrane proteins (nodulin-like) were identified. These may represent target genes responsible for vesicular transport and the fusion with the vacuoles.

Together, these results confirmed VvMYB5a, VvMYB5b and VvMYBA1 as regulators of flavonoid pathway, even if only VvMYBA1 regulates the late steps for the anthocyanin production. Moreover, VvMYB5a may control also the lignin synthesis. The analyses provided also indications of VvMYB5a and VvMYB5b involvement in vehicles transport to the central vacuole.

Table 6. Up-regulated genes in *35S::VvMYBA1*, *35S::VvMYB5a* and *35S::VvMYB5b* petals compared to the untransformed line *an2*.

SEQ_ID	**GENE DESCRIPTION**	**FC VvMYBA1**	**FC VvMYB5a**	**FC VvMYB5b**
PETAX062758_Contig1_314_39_S	Xyloglucan endotransglucosylase-hydrolase *(Solanum lycopersicum)*	4.1	3.1	-
PETAX026374_Contig1_274_38_S	no hit	3.1	2.8	-
PETAX064323_Contig2_404_40_S	no hit	6.5	6.2	-
PETAX064323_Contig1_241_39_X2	no hit	2.9	3.6	-
PETAX087824_Contig3_164_35_X4	no hit	65.2	-	3.2
PETAX062686_Contig1_153_35_X2	no hit	48.3	-	3.5
PETAX087824_Contig5_224_36_X2	no hit	39.7	-	3.0
PETAX025229_Contig1_295_40_X4	Serine carboxypeptidase *(Ricinus communis)*	21.2	-	2.5
PETAX030315_Contig1_73_36_S	no hit	14.1	-	2.1
PETAX060521_Contig1_487_37_S	Serine carboxypeptidase *(Ricinus communis)*	13.2	-	12.2
PETAX012531_Contig1_485_40_S	Predicted protein *(Populus trichocarpa)*	11.4	-	3.7
PETAX087824_Contig1_144_36_X2	no hit	11.0	-	2.6
PETAX014375_Contig2_266_40_X2	no hit	10.4	-	4.5
PETAX066446_Contig1_373_40_S	Cytochrome B5 *(Petunia hybrida)*	9.7	-	5.4
PETAX014375_Contig1_504_39_X2	no hit	6.6	-	4.1
PETAX006470_Contig1_572_35_S	Anthocyanidin synthase *(Ipomoea purpurea)*	6.2	-	3.4
PETAX062423_Contig1_206_35_S	Dihydroflavonol-4-reductase *(Petunia hybrida)*	5.0	-	6.7
PETAX053498_Contig1_373_40_S	Mycolic acid methyl transferase-like protein *(Arabidopsis thaliana)*	4.8	-	2.8
PETAX090363_Contig1_305_40_S	Dihydroflavonol-4-reductase *(Petunia hybrida)*	4.3	-	5.7
PETAX006361_Contig2_12_40_S	Unknown protein *(Nicotiana tabacum*)	3.0	-	3.8
PETAX015426_Contig1_418_40_S	Unknown protein *(Vitis vinifera* GSVIVT00030722001)	2.5	-	3.8
PETAX086238_Contig1_331_40_S	no hit	2.5	-	2.3
PETAX008423_Contig1_377_40_S	no hit	2.3	-	2.2
PETAX006361_Contig1_456_40_S	Unknown protein *(Nicotiana tabacum)*	2.3	-	3.0
PETAX001318_Contig2_637_37_S	no hit	12.2	2.5	2.5
PETAX071872_Contig1_575_39_S	Unknown protein (*Vitis vinifera* GSVIVT00011405001)	-	8.5	3.8
PETAX014081_Contig2_457_40_S	Lipid-transfer protein *(Nicotiana attenuata)*	-	8.5	2.2
PETAX088705_Contig1_191_39_S	no hit	-	5.1	2.7
PETAX037256_Contig1_400_40_S	no hit	-	4.6	3.7
PETAX054602_Contig1_70_40_S	Hf2 gene for flavonoid 3', 5'-hydroxylase *(Petunia integrifolia*)	-	4.3	2.8
PETAX020993_Contig1_332_39_S	no hit	-	2.7	3.7
PETAX007193_Contig1_375_39_S	Unknown protein (*Nicotiana tabacum* TT-06_I15)	-	2.7	4.1

DISCUSSION

In this work, we used *Petunia hybrida* as a model system to study the functions of the grape transcription factors VvMYB5a and VvMYB5b. To date, the information available was provided by heterologous expression in tobacco and tomato revealing their involvement in the regulation of the early steps of the flavonoid pathway (Deluc *et al.*, 2006; Deluc *et al.*, 2008; Mahjoub *et al.*, 2009). Here we based our studies on the high similarity of VvMYB5a and VvMYB5b with the petunia PH4, regulator of vacuolar pH and vesicle transport in epidermal cells of petals (Quattrocchio *et al.*, 2006; Faraco, 2011). The *ph4* mutation, previously characterized by Quattrocchio *et al.* (2006) affects the acidification of the lumen where the anthocyanin pigments are stored with a shift of petal colour from red to violet-blue. The use of *ph4* petunia mutant to perform heterologous expression was useful to demonstrate that VvMYB5a and VvMYB5b are functional homolog to PhPH4. In fact, the constitutive expression in *ph4* background induced fully complementation of the mutant phenotype. Both transgenic plants presented pink-reddish pigmentation due to lower pH values of petal extracts in comparison to the *ph4* untransformed line. The restoration of wild type acidic pH values was likely due to the induction of the structural genes of the acidification pathway, that we demonstrated for *PhCAC 16.5* and *PhPH5*.

As previously shown by Deluc, the expression of *VvMYB5a* and *VvMYB5b* in tobacco plants enhances the pigmentation in petals and stamens. The heterologous expression of both genes in petunia *ph4* mutant caused no change in anthocyanin content and profile and, indeed, the expression of structural genes for flavonoid pathway in petals was not modified. Only expressing *VvMYB5a* and *VvMYB5b* in a petunia line mutated in *PhAN2* locus and lacking of anthocyanins, we could confirm their ability to positively regulate the flavonoid structural genes. The increase of pigmentation observed in petals of transgenic *an2* plants was similar to that of *an2* plant transformed with *35S:PhPH4* (unpublished data). In fact, the use of a petunia line mutated for a specific MYB ensures that VvMYB5a and VvMYB5b, when expressed constitutively, could overcome the lack of this MYB factor in the complex WD40-bHLH-MYB and perform their regulatory functions. In *ph4* mutant background, VvMYB5a and VvMYB5b could only substitute PhPH4 in the regulation of vacuolar acidification; we could not observe their involvement in the flavonoid pathway because of possible overlapping functions with the endogenous wild type PhAN2. For this reason, the use of mutant lines for heterologous expression is helpful to obtain useful information and to verify specific gene functions.

Petunia *an2* plants expressing *VvMYB5a* and *VvMYB5b* presented different pigmentation and colour intensity in comparison to plants transformed with *VvMYBA1* and this could be due, at least in part, to specific ability of VvMYB5a and VvMYB5b in the regulation of vacuolar acidification. Their involvement in the vacuolar acidification could be verified also in *an2* plants because the mutation in *AN2* locus causes a weak reduction of *PhPH5* expression, partially affecting the vacuolar pH (Quattrocchio *et al.*, 1999; Verweij *et al.*, 2008).

In addition, these MYB factors differently regulate the anthocyanin structural genes. In grapevine, VvMYB5a and VvMYB5b control several enzymes of the general flavonoid pathway, except the step catalyzed by the UFGT (UDP-Glc:flavonoid 3-O-glucosyltransferase) which is under the control of VvMYBA1. In petunia the regulatory mechanism is different, because the anthocyanin specific MYB protein PhAN2 regulates all the enzymatic steps from the reduction of dihydroflavonols to leucoanthocyanidins, catalyzed by DFR onwards. This depends on the promoter region of these late structural genes that are recognized by the WD40-bHLH-MYB complex driven by PhAN2 or an ortholog from another species, as confirmed by the *an2* petunia plants expressing *VvMYBA1*. Our findings suggests that in *an2* petunia, VvMYB5a and VvMYB5b act like the endogenous PhAN2 by regulating a subset of late enzymatic steps. In fact, as PhAN2, they activate for example the expression of Ph*DFR*, but they seem unable to control enzymes involved in latest steps of the anthocyanin synthesis such as acylation and glycosilation, or the transport to the vacuole mediated by PhAN9. When expressed in tobacco, VvMYB5a and VvMYB5b induced only the expression of the early anthocyanin genes *CHS*, *CHI*, and *F3H* in petals. In contrast with our results, *DFR* expression appeared down-regulated. The regulative mechanism for the flavonoid synthesis is still poorly studied in tobacco, so it is difficult comparing the different results obtained by heterologous expression in the two species.

While the impact of *VvMYB5a* and *VvMYB5b* expression on anthocyanin production is apparent only in petunia petals, VvMYBA1 induces pigment accumulation also in other organs such as leaves. This possibly reflects the dependence on a bHLH partner. Because the bHLH *PhAN1* is expressed only in petals (Spelt *et al.*, 2000), the pigmentation in different organs of *VvMYBA1* plants could be explained by the specific ability of VvMYBA1 to activate the expression of the endogenous *PhAN1*. Expression analysis showed that similarly to PhAN2 (Spelt *et al.*, 2000), VvMYBA1 is able to induce *PhAN1* expression. A weak induction was observed only in *VvMYB5b* expressing leaves.

In order to clarify the specific roles of VvMYB5a and VvMYB5b and to compare their functions to VvMYBA1 role, we analyzed the transcriptomic variations induced by their expression in petunia *an2* mutant line. The analyses underlined both specific and common genes modulated by the expression of the three MYB factors. Genes modulated by VvMYB5a and VvMYB5b are almost completely distinct from those up- or down-regulated by VvMYBA1 and only few of them are related to the flavonoid pathway. In fact, VvMYB5b can regulate the late structural genes, but none of the enzymes responsible for anthocyanin modifications and transport, that resulted instead modulated by VvMYBA1, whose involvement in anthocyanin branch was already provided by Cutanda-Perez *et al.* in grapevine (2009). In addition to the flavonoid genes, the microarray analysis revealed that VvMYBA1 induced many other genes whose function is still unknown, suggesting the involvement in the regulation of other metabolic processes. Regarding *VvMYB5a* expression, we observed the induction of a set of genes putatively involved in the lignin pathway, as *CCoAOMT* gene that plays a fundamental role for lignin accumulation and composition in plants (Zhong *et al.*, 1998). However, this result is inconsistent with the previous results obtained in tobacco. It is possible that in a wild type line *VvMYB5a* expression might compete with the endogenous MYB factor responsible for lignin synthesis, resulting in a decrease of structural gene expression. To reduce the risk of competition, we used petunia line mutated for the expression of endogenous MYB factors. Further phenotypic analysis of the transgenic petunia plants will provide more information about the role of VvMYB5a in the regulation of lignin pathway.

Our transcriptomic data suggest that VvMYB5a and VvMYB5b may be involved in the control of multiple processes. Both of them drive the expression of some lipid-transfer proteins and membrane proteins, hypothetically involved in the vesicular transport and fusion to the central vacuoles. In petunia, in addition to the pH structural genes, PhPH4 and PhPH3 regulate another set of downstream genes important for the formation of an intermediate cellular compartment called vacuolino that deliveries proteins to the central vacuoles (Faraco, 2011). The H^+ pumps PhPH1 and PhPH5 located on the tonoplast could mediate the fusion to the vacuole interacting with SNARE proteins on the vacuolino membrane (Verweij *et al.*, 2008; Faraco, 2011; Provenzano, 2011). The forced expression of *VvMYB5a* and *VvMYB5b* could result in a different regulation of *PhPH1* and *PhPH5* genes, leading to altered concentrations of ions inside the lumen and a change of electrical gradient across the tonoplast. Consequently, differences in cell dimensions observed in petals of *VvMYB5a* and *VvMYB5b* expressing plants could be explained by different dimension of vacuole or vacuole-structures. However, further analyses are necessary to confirm the role of VvMYB5a and VvMYB5b in the sorting of vacuolar proteins. Changes in cell shape and dimensions can be alternatively due to the pigment accumulation, as assessed in petunia mutants (van Houwelingen, 1998) and this is consistent with the role of flavonoids in modulating polar auxin transport (Peer and Murphy, 2007). Another possibility is that VvMYB5a and VvMYB5b regulate also processes that contribute to the final cell shape, as a MYB-like gene homologous to MIXTA from snapdragon (Noda *et al.*, 1994; Baumann *et al.*, 2007). Other questions arise from these hypotheses, blazing new trails for research in the regulation of the vacuole biogenesis also in grapevine.

Previous phylogenetic analyses showed that VvMYB5a and VvMYB5b belong to a small cluster that includes other four MYB proteins involved in the control of various physiological and developmental processes (Deluc *et al.*, 2008). It is therefore plausible that the roles of VvMYB5a and VvMYB5b in grapevine could be other than vacuolar acidification. Therefore, it is still unclear if the information obtained in this work reflects the real situation in the native species. Further work in the homologous system is therefore required to determine the precise function of VvMYB5a and VvMYB5b.

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SUPPLEMENTARY DATA

Supplementary table 1. Differentially expressed genes (> |2| fold) in *VvMYB5b* expressing flowers compared to the untransformed line.

SEQ_ID	GENE DESCRIPTION	FC
PETAX006060_Contig1_540_40_S	no hit	33.8
PETAX078379_Contig1_297_40_S	RNase Phy4 *(Petunia hybrida*)	24.7
PETAX060521_Contig1_487_37_S	Serine carboxypeptidase *(Ricinus communis)*	12.2
PETAX031168_Contig1_435_40_S	Endochitinase *(Capsicum annuum)*	11.0
PETAX046579_Contig1_286_40_S	3'-N-debenzoyl-2'-deoxytaxol N-benzoyltransferase *(Ricinus communis)*	8.8
PETAX071872_Contig1_575_39_S	Unknown protein (*Vitis vinifera* GSVIVT00011405001)	8.5
PETAX014081_Contig2_457_40_S	Lipid-transfer protein *(Nicotiana attenuata)*	8.5
PETAX062423_Contig1_206_35_S	Dihydroflavonol-4-reductase *(Petunia hybrida)*	6.7
PETAX089413_Contig1_294_40_S	Zinc binding dehydrogenase *(Ricinus communis)*	5.8
PETAX090363_Contig1_305_40_S	Dihydroflavonol-4-reductase *(Petunia hybrida)*	5.7
PETAX068373_Contig1_359_38_S	Polyphosphoinositide binding protein *(Arabidopsis thaliana)*	5.5
PETAX066446_Contig1_373_40_S	Cytochrome B5 *(Petunia hybrida)*	5.4
PETAX088705_Contig1_191_39_S	no hit	5.1
PETAX069683_Contig1_325_36_S	Unknown protein (*Vitis vinifera* GSVIVT00035108001)	5.0
PETAX037256_Contig1_400_40_S	no hit	4.6
PETAX014375_Contig2_266_40_X2	no hit	4.5
PETAX054602_Contig1_70_40_S	Hf2 gene for flavonoid 3'. 5'-hydroxylase *(Petunia integrifolia* var. integrifolia)	4.3
PETAX077682_Contig1_200_40_S	Acyltransferase *(Capsicum frutescens)*	4.2
PETAX027410_Contig1_340_39_S	Unknown protein *(Vitis vinifera* GSVIVT00003016001)	4.2
PETAX014375_Contig1_504_39_X2	no hit	4.1
PETAX001393_Contig2_69_40_S	Sucrose synthase 4 *(Solanum tuberosum)*	3.9
PETAX006361_Contig2_12_40_S	Unknown protein *(Nicotiana tabacum)*	3.8
PETAX015426_Contig1_418_40_S	Unknown protein (*Vitis vinifera* GSVIVT00030722001)	3.8
PETAX012531_Contig1_485_40_S	Predicted protein *(Populus trichocarpa)*	3.7
PETAX043185_Contig2_206_38_S	Predicted protein *(Populus trichocarpa)*	3.6
PETAX068472_Contig1_430_39_S	Autoinhibited calcium ATPase *(Populus trichocarpa)*	3.5
PETAX005440_Contig1_256_40_S	Purine transporter *(Ricinus communis)*	3.5
PETAX062686_Contig1_153_35_X2	no hit	3.5
PETAX006470_Contig1_572_35_S	Anthocyanidin synthase *(Ipomoea purpurea)*	3.4
PETAX090339_Contig1_257_40_S	Mtn21-like protein *(Populus trichocarpa)*	3.4
PETAX039849_Contig1_415_37_S	no hit	3.2
PETAX087824_Contig3_164_35_X4	no hit	3.2
PETAX071176_Contig1_459_39_S	CAPRICE *(Gossypium herbaceum)*	3.2
PETAX028060_Contig1_248_40_S	no hit	3.1
PETAX052935_Contig1_87_40_S	Unknown protein (*Solanum lycopersicum* clone LEFL1007CG10)	3.1
PETAX006361_Contig1_456_40_S	Unknown protein (*Nicotiana tabacum*)	3.0
PETAX087824_Contig5_224_36_X2	no hit	3.0
PETAX042158_Contig1_390_40_S	Unknown protein (*Vitis vinifera* GSVIVT00024986001)	3.0
PETAX030027_Contig1_95_39_X2	Unknown protein (*Solanum lycopersicum* clone LEFL2011M04)	2.8
PETAX055565_Contig1_611_40_S	Protein phosphatase 2c *(Ricinus communis)*	2.8
PETAX083618_Contig1_224_40_S	Malic enzyme *(Solanum lycopersicum)*	2.8
PETAX053498_Contig1_373_40_S	Mycolic acid methyl transferase-like protein *(Arabidopsis thaliana)*	2.8
PETAX020993_Contig1_332_39_S	no hit	2.7
PETAX007193_Contig1_375_39_S	Unknown protein (*Nicotiana tabacum* TT-06_I15)	2.7
PETAX081423_Contig1_304_35_S	no hit	2.7
PETAX050811_Contig1_453_39_S	Putative uncharacterized protein *(Ricinus communis)*	2.7
PETAX087824_Contig1_144_36_X2	no hit	2.6
PETAX025229_Contig1_295_40_X4	Serine carboxypeptidase (*Ricinus communis*)	2.5
PETAX015268_Contig1_183_40_S	Unknown protein (*Solanum lycopersicum* clone LEFL1007CG10)	2.5
PETAX062495_Contig1_471_40_S	EIX receptor 2 *(Solanum lycopersicum)*	2.5
PETAX001318_Contig2_637_37_S	no hit	2.5
PETAX042463_Contig1_343_40_S	Unknown protein (*Vitis vinifera* GSVIVT00033997001)	2.4
PETAX016239_Contig2_328_40_S	Catechol o-methyltransferase *(Populus trichocarpa)*	2.4
PETAX000472_Contig2_311_40_X2	Delta-1-pyrroline-5-carboxylate synthetase *(Saccharum arundinaceum)*	2.4
PETAX028193_Contig1_597_40_S	Putative uncharacterized protein *(Nicotiana tabacum*)	2.4
PETAX034903_Contig1_421_39_S	P70 protein *(Nicotiana tabacum)*	2.3
PETAX030027_Contig2_565_39_S	Unknown protein (*Vitis vinifera* GSVIVT00020560001)	2.3
PETAX086238_Contig1_331_40_S	no hit	2.3
PETAX008809_Contig1_821_40_S	Putative uncharacterized protein *(Solanum lycopersicum)*	2.3
PETAX094459_Contig1_353_35_S	no hit	2.3
PETAX027757_Contig1_315_40_S	Predicted protein *(Populus trichocarpa)*	2.2
PETAX091469_Contig1_430_38_S	no hit	2.2
PETAX008423_Contig1_377_40_S	no hit	2.2
PETAX076325_Contig1_611_40_S	Putative uncharacterized protein *(Populus trichocarpa)*	2.2
PETAX050203_Contig1_412_40_S	Kinesin heavy chain-like protein *(Solanum tuberosum)*	2.2
PETAX076560_Contig1_469_40_S	GATA transcription factor *(Ricinus communis)*	2.1
PETAX059331_Contig1_443_40_S	Putative uncharacterized protein *(Populus trichocarpa)*	2.1
PETAX030315_Contig1_73_36_S	no hit	2.1
PETAX059678_Contig1_65_35_S	Putative non-specific lipid transfer protein *(Solanum tuberosum)*	2.1
PETAX029081_Contig1_445_39_S	Phosphoenolpyruvate carboxylase *(Ricinus communis)*	2.0
PETAX098820_Contig1_63_35_S	no hit	2.0

Supplementary table 2. Differentially expressed genes (> |2| fold) in *VvMYBA1* expressing flowers compared to the untransformed line.

SEQ_ID	GENE DESCRIPTION	FC
PETAX085370_Contig1_320_39_S	no hit	61.2
PETAX062686_Contig1_153_35_X2	no hit	48.2
PETAX087824_Contig5_224_36_X2	no hit	39.7
PETAX024978_Contig2_286_40_S	no hit	30.9
PETAX066815_Contig2_692_39_S	UDP rhamnose:anthocyanidin-3-glucoside rhamnosyltransferase *(Petunia axillaris)*	30.5
PETAX027976_Contig1_285_40_S	no hit	28.7
PETAX007368_Contig1_256_40_S	Glutathione S-transferase *(Petunia hybrida)*	28.1
PETAX002242_Contig1_360_38_X2	no hit	24.5
PETAX024978_Contig1_381_36_S	no hit	22.2
PETAX025229_Contig1_295_40_X4	Serine carboxypeptidase *(Ricinus communis)*	21.2
PETAX080846_Contig1_125_35_X2	no hit	18.5
PETAX025229_Contig2_28_40_S	Serine carboxypeptidase *(Ricinus communis)*	18.3
PETAX002242_Contig3_255_35_X2	no hit	16.3
PETAX025360_Contig1_392_40_S	no hit	15.9
PETAX100402_Contig1_88_40_S	no hit	15.2
PETAX030315_Contig1_73_36_S	no hit	14.1
PETAX080846_Contig2_13_38_S	no hit	13.5
PETAX060521_Contig1_487_37_S	Serine carboxypeptidase *(Ricinus communis)*	13.2
PETAX091494_Contig1_215_40_S	no hit	13.1
PETAX006874_Contig1_415_40_S	no hit	12.8
PETAX001318_Contig2_637_37_S	no hit	12.2
PETAX080933_Contig1_417_38_S	Anthocyanin 5-O-glucosyltransferase *(Petunia hybrida)*	12.0
PETAX012531_Contig1_485_40_S	Predicted protein *(Populus trichocarpa)*	11.3
PETAX087824_Contig1_144_36_X2	no hit	10.9
PETAX001137_Contig2_206_40_X2	no hit	10.4
PETAX031145_Contig1_439_39_S	Caffeoyl-CoA O-methyltransferase (*Plantago major*)	10.4
PETAX014375_Contig2_266_40_X2	no hit	10.3
PETAX006899_Contig2_29_38_S	no hit	10.3
PETAX066446_Contig1_373_40_S	Cytochrome B5 *(Petunia hybrida)*	9.7
PETAX030315_Contig2_523_40_S	no hit	9.7
PETAX060754_Contig1_309_35_S	no hit	9.0
PETAX024608_Contig1_473_39_S	no hit	8.4
PETAX036148_Contig1_74_40_S	no hit	8.3
PETAX055274_Contig1_364_40_S	Heat shock protein *(Ricinus communis)*	8.2
PETAX016253_Contig1_465_39_S	Putative anthocyanin permease *(Solanum lycopersicum)*	7.7
PETAX001137_Contig1_215_35_S	no hit	7.3
PETAX031631_Contig1_350_39_S	no hit	7.2
PETAX024227_Contig1_18_39_S	no hit	7.0
PETAX014375_Contig1_504_39_X2	no hit	6.6
PETAX080314_Contig1_345_40_S	no hit	6.5
PETAX064323_Contig2_404_40_S	no hit	6.4
PETAX087824_Contig6_189_35_X2	no hit	6.3
PETAX047068_Contig1_342_39_S	Anthocyanin acyltransferase *(Solanum lycopersicum)*	6.3
PETAX006470_Contig1_572_35_S	Anthocyanidin synthase *(Ipomoea purpurea)*	6.1
PETAX001318_Contig1_443_39_S	no hit	5.8
PETAX005182_Contig1_411_37_S	Cytochrome P450 monooxygenase *(Petunia hybrida)*	5.7
PETAX029181_Contig1_408_40_S	SUMO *(Nicotiana benthamiana)*	5.6
PETAX011516_Contig1_398_35_S	no hit	5.5
PETAX028168_Contig1_267_40_S	Putative uncharacterized protein (*Oryza sativa* subsp. Indica)	5.4
PETAX026649_Contig2_328_40_S	Cytosolic class I small heat shock protein 1A (*Nicotiana tabacum)*	5.2
PETAX016253_Contig2_363_40_X2	Putative anthocyanin permease *(Solanum lycopersicum)*	5.2
PETAX062423_Contig1_206_35_S	Dihydroflavonol-4-reductase *(Petunia hybrida)*	5.0
PETAX053498_Contig1_373_40_S	Mycolic acid methyl transferase-like protein *(Arabidopsis thaliana)*	4.8
PETAX095100_Contig1_369_40_S	no hit	4.7
PETAX087354_Contig1_397_40_S	Heat shock protein 83 (*Beta vulgaris*)	4.5
PETAX090363_Contig1_305_40_S	Dihydroflavonol-4-reductase *(Petunia hybrida)*	4.3
PETAX062758_Contig1_314_39_S	Xyloglucan endotransglucosylase-hydrolase XTH3 *(Solanum lycopersicum)*	4.0
PETAX047889_Contig1_398_40_S	no hit	4.0
PETAX024227_Contig2_17_35_S	no hit	3.7
PETAX000887_Contig1_312_40_X2	ER luminal-binding protein *(Nicotiana benthamiana)*	3.7
PETAX005157_Contig1_636_40_S	Anthocyanidin 3-O-glucosyltransferase *(Petunia hybrida)*	3.6
PETAX011953_Contig1_132_40_S	101 kDa heat shock protein *(Nicotiana tabacum)*	3.5
PETAX017948_Contig1_547_36_S	Heat shock protein *(Ricinus communis)*	3.4
PETAX006899_Contig1_360_38_X2	no hit	3.4
PETAX009021_Contig1_231_40_X2	Unknown protein (*Vitis vinifera* GSVIVT00011129001)	3.3
PETAX000671_Contig2_302_40_X2	Molecular chaperone Hsp90-2 *(Nicotiana benthamiana)*	3.1
PETAX028412_Contig1_350_35_S	Multiprotein bridging factor ER24 *(Solanum lycopersicum)*	3.1
PETAX000142_Contig2_303_40_X2	Gamma-thionin *(Nicotiana excelsior)*	3.0
PETAX026374_Contig1_274_38_S	no hit	3.0
PETAX000887_Contig2_124_35_S	ER luminal-binding protein *(Nicotiana benthamiana)*	2.9
PETAX006361_Contig2_12_40_S	Unknown protein *(Nicotiana tabacum*)	2.9
PETAX092308_Contig1_355_40_X2	no hit	2.9
PETAX016417_Contig1_212_40_X3	Phi-1 protein *(Nicotiana tabacum)*	2.9
PETAX064323_Contig1_241_39_X2	no hit	2.9
PETAX029373_Contig1_348_40_X2	Phi-1 protein *(Nicotiana tabacum)*	2.9
PETAX053395_Contig1_330_35_S	no hit	2.8
PETAX031248_Contig1_422_40_S	no hit	2.8
PETAX026948_Contig1_262_40_X2	S2 self-incompatibility ribonuclease (S2-RNase) (*Petunia integrifolia* subsp. Inflata)	2.8
PETAX002192_Contig2_325_37_X2	Putative heat-shock protein 90 *(Euphorbia esula)*	2.7
PETAX002192_Contig1_559_39_S	Putative heat-shock protein 90 *(Euphorbia esula)*	2.7
PETAX022265_Contig1_621_39_S	ER luminal-binding protein *(Nicotiana benthamiana)*	2.7
PETAX051276_Contig1_469_40_S	Unknown protein (*Vitis vinifera* GSVIVT00019227001)	2.6
PETAX035978_Contig2_488_40_S	Heat shock protein 60 *(Ageratina adenophora)*	2.6
PETAX073178_Contig2_331_38_X2	Unknown protein (*Lycopersicon esculentum* clone 133278F)	2.6
PETAX001100_Contig1_629_38_S	Putative uncharacterized protein *(Solanum lycopersicum)*	2.6
PETAX011919_Contig1_648_36_S	Unknown protein (*Vitis vinifera* GSVIVT00001871001)	2.5
PETAX001656_Contig1_638_39_S	no hit	2.5
PETAX047598_Contig1_420_40_S	Predicted protein *(Populus trichocarpa)*	2.5
PETAX008822_Contig1_238_40_S	Phi-1 protein *(Nicotiana tabacum)*	2.5

PETAX015426_Contig1_418_40_S	Unknown protein (*Vitis vinifera* GSVIVT00030722001)	2.5
PETAX081901_Contig1_340_37_S	no hit	2.4
PETAX001170_Contig1_32_40_S	nematode resistance-like protein (Gro1-3) *(Solanum tuberosum)*	2.4
PETAX086238_Contig1_331_40_S	no hit	2.4
PETAX073178_Contig1_251_35_X2	Unknown protein (*Vitis vinifera* GSVIVT00022204001)	2.4
PETAX061900_Contig1_311_40_S	Putative uncharacterized protein (*Picea sitchensis)*	2.4
PETAX025495_Contig1_588_37_S	Unknown protein (*Vitis vinifera* GSVIVT00023265001)	2.4
PETAX076974_Contig1_437_39_S	Phosphosulfolactate synthase-related protein *(Solanum lycopersicum)*	2.4
PETAX030083_Contig1_279_35_S	GRAS10 *(Solanum lycopersicum)*	2.4
PETAX023371_Contig1_165_38_X2	no hit	2.4
PETAX072696_Contig1_112_40_S	Putative transposase (*Petunia integrifolia* subsp. Inflata)	2.4
PETAX014871_Contig1_385_40_S	Early endosome antigen *(Ricinus communis)*	2.3
PETAX003808_Contig1_779_40_S	Endoplasmin *(Ricinus communis)*	2.3
PETAX097165_Contig1_283_40_S	Predicted protein *(Populus trichocarpa)*	2.3
PETAX027076_Contig2_179_38_X2	no hit	2.3
PETAX073178_Contig3_681_40_S	Predicted protein *(Populus trichocarpa)*	2.3
PETAX018446_Contig1_429_40_S	Hsp20/alpha crystallin family protein *(Solanum demissum)*	2.3
PETAX008423_Contig1_377_40_S	no hit	2.2
PETAX006361_Contig1_456_40_S	Unknown protein *(Nicotiana tabacum)*	2.2
PETAX004664_Contig1_417_39_S	Unknown protein (*Vitis vinifera* GSVIVT00003050001)	2.2
PETAX055544_Contig1_177_40_X2	S2 self-incompatibility ribonuclease (S2-RNase) (*Petunia integrifolia* subsp. Inflata)	2.2
PETAX005667_Contig2_438_38_S	PCRTC *(Capsicum annuum)*	2.2
PETAX006016_Contig1_340_40_X2	NtSar1 protein-like *(Solanum tuberosum)*	2.2
PETAX022455_Contig3_434_36_X2	Unknown protein (*Solanum lycopersicum* LEFL1064BH10)	2.1
PETAX021604_Contig2_487_38_S	Heat shock protein *(Ricinus communis)*	2.1
PETAX027395_Contig1_577_40_S	Zinc finger protein *(Fagus sylvatica)*	2.1
PETAX013024_Contig3_367_40_S	Unknown protein (*Vitis vinifera* GSVIVT00015029001)	2.1
PETAX024239_Contig1_639_40_S	Adenosine 3'-phospho 5'-phosphosulfate transporter 1 *(Solanum demissum)*	2.1
PETAX060397_Contig1_222_35_S	Beta-glucosidase *(Solanum lycopersicum)*	2.1
PETAX016417_Contig2_23_35_S	Phi-1 protein *(Nicotiana tabacum)*	2.1
PETAX024527_Contig1_523_35_S	Nucellin-like protein *(Daucus carota)*	2.1
PETAX008815_Contig1_545_40_S	Unknown protein (*Vitis vinifera* GSVIVT00015269001)	2.1
PETAX048946_Contig1_324_39_S	Putative DNAJ protein *(Nicotiana tabacum)*	2.1
PETAX002399_Contig1_676_37_S	Putative uncharacterized protein *(Picea sitchensis)*	2.1
PETAX000752_Contig1_524_40_S	P58IPK *(Nicotiana benthamiana)*	2.1
PETAX006652_Contig1_704_37_S	60S ribosomal protein *(Populus trichocarpa)*	2.1
PETAX005667_Contig1_382_39_X2	PCRTC *(Capsicum annuum)*	2.1
PETAX027399_Contig1_295_35_X2	Putative UDP-galactose transporter *(Solanum demissum)*	2.1
PETAX053401_Contig1_237_40_S	Aspartic proteinase Asp1 *(Ricinus communis)*	2.1
PETAX025679_Contig1_621_35_S	Acyl carrier protein *(Solanum tuberosum)*	2.1
PETAX052109_Contig1_572_39_S	Unknown protein (*Vitis vinifera* GSVIVT00000039001)	2.0
PETAX096461_Contig1_430_40_S	Unknown protein (*Nicotiana tabacum* clone nt002224008)	2.0
PETAX027076_Contig4_336_38_S	no hit	2.0
PETAX023169_Contig3_320_38_X2	Unknown protein (*Solanum lycopersicum* LEFL1079BG06)	2.0
PETAX016559_Contig1_458_38_S	Metal transport protein *(Medicago truncatula)*	2.0
PETAX031804_Contig1_554_37_S	no hit	2.0
PETAX021133_Contig1_526_36_S	Putative 7-transmembrane G-protein-coupled receptor *(Solanum chacoense)*	2.0
PETAX053870_Contig1_310_35_S	S self-incompatibility ribonuclease (S2-RNase) (*Petunia integrifolia* subsp. Inflata)	2.0
PETAX043204_Contig1_312_39_S	Unknown protein (*Nicotiana tabacum* clone KL4B.102M17)	2.0
PETAX002352_Contig1_732_40_S	Putative uncharacterized protein *(Populus trichocarpa)*	2.0
PETAX050292_Contig1_482_39_S	no hit	2.0
PETAX022455_Contig5_330_39_X5	Unknown protein (*Solanum lycopersicum* LEFL1064BH10)	2.0
PETAX001757_Contig1_504_35_S	Unknown protein (*Vitis vinifera* GSVIVT00035302001)	2.0
PETAX013024_Contig2_292_40_X2	Unknown protein (*Solanum lycopersicum* LEFL1002AG04)	2.0
PETAX013076_Contig1_501_39_S	Hydrolase *(Ricinus communis)*	2.0
PETAX051411_Contig1_444_40_S	Salt tolerance protein 5-like protein *(Solanum tuberosum)*	2.0

Chapter 3

VvMYB5a and VvMYB5b regulate multiple metabolic pathways in addition to flavonoid biosynthesis in *Vitis vinifera*

ABSTRACT

MYB proteins represent a class of transcription factors widely distributed in plant kingdom and involved in the regulation of numerous physiological processes. In grapevine, the MYB factors studied so far are mainly related to the flavonoid biosynthesis, as the recently identified VvMYB5a and VvMYB5b, the orthologs of *Petunia hybrida* PH4, that regulates vacuolar acidification in petal epidermis (Quattrocchio *et al.*, 2006) and *Arabidopsis thaliana* MYB5, involved in the regulation of trichome development, proanthocyanidin synthesis and mucilage deposition in seed coat (Li *et al.*, 2009). *VvMYB5a* and *VvMYB5b* are expressed similarly in many vegetative organs, while in berry their expression profiles are divergent. To date, the information available about their putative regulative functions in grapevine have been mainly inferred by heterologous expression in tobacco (Deluc *et al.*, 2006; Deluc *et al.*, 2008), tomato (Mahjoub *et al.*, 2009) and petunia (Chapter 2). Here we describe the functional analyses of VvMYB5a and VvMYB5b directly in the native species *Vitis vinifera*, using two different approaches: ectopic expression in hairy roots and stable transformation of embryogenic callus. Our results suggest that they are not only involved in the regulation of the flavonoid pathway but also in the control of other metabolic and developmental processes.

INTRODUCTION

The MYB proteins represent one of the largest families of transcription factors common to all eukaryotes. The first MYB protein identified in plants was C1 involved in the regulation of flavonoid synthesis in mais (Paz-Ares *et al.*, 1987). Afterwards, MYB proteins have been investigated in numerous plant species such as Arabidopsis, maize, rice and petunia, where they are involved in a wide variety of processes such as embryogenesis, circadian clock regulation, flowering time, light signal transduction, trichome development, shoot morphogenesis, lignification, dehydration and ABA response (Li *et al.*, 1996; Jin and Martin, 1999; Newman *et al.*, 2004).

The common feature of all MYB factors is the presence of a DNA-binding domain at the N-terminus. It consists of up to four imperfect sequence repeats (R) of about 52 amino acids, each forming a helix-turn-helix conformation. Depending on the number of adjacent repeats, MYB proteins can be divided into four groups (1R-MYB, R2R3MYB, 3R-MYB and 4R-MYB). Although all classes are found in plants, R2R3-MYB proteins, containing two MYB repeats, represent the largest group of plant MYB factors.

In *Vitis vinifera*, the R2R3 MYB family is composed of 108 members as revealed by a wide genome analysis performed by Matus *et al*. (2008). Most of the MYB transcription factors so far characterized regulate specifically the production of flavonoids involved in development and adaptation to the environment. These secondary metabolites are also important for wine quality and conservation, contributing to the organoleptic qualities such as colour, taste and flavour. Proanthocyanidins (PA), synthesized in seeds and skin of the fruit during the early stages of development, are responsible for the bitterness and astringency of wine; anthocyanins are the principal pigments accumulated in berry skin of red cultivars during the ripening; flavonols are produced mainly in flowers and skins of grape berries where they can affect anthocyanin colour in wine.

The synthesis of each flavonoid compound occurs in specific tissues at different stages of berry development suggesting that the expression of structural genes involved in the biosynthetic pathway is highly coordinated. As already demonstrated in other species, this regulatory mechanism relies on WD40-bHLH-MYB complex where the MYB transcription factors play a fundamental role determing the specificity of the process regulated. For example, two MYB transcription factors recently characterized, VvMYBPA1 and VvMYBPA2 (Bogs *et al.*, 2007; Terrier *et al.*, 2009) regulate the enzymes common to all flavonoids, such as VvCHI, VvF3'5'H and VvANS as well as VvLAR1 and VvANR specific for PA branch. VvMYBA1 (Kobayashi *et al.*, 2002; Kobayashi *et al.*, 2004) and VvMYBA2 (Bogs *et al.*, 2007; Walker *et al.*, 2007) direct only the anthocyanin synthesis through the coordinated expression of *VvUFGT*. VvMYBF1 was identified as the transcriptional regulator of *VvFLS1* for flavonol synthesis (Czemmel *et al.*, 2009). Recently, two MYB transcription factors named VvMYB5a and VvMYB5b were identified. They belong to a small cluster of R2R3-MYB transcription factors involved in the control of various physiological and developmental processes, as the petunia PH4, regulator of vacuolar acidification in epidermis of flowers (Quattrocchio *et al.*, 2006) and Arabidopsis MYB5 that controls seed coat differentiation, trichome development, tannin production and mucilage production (Gonzalez *et al.*, 2009; Li *et al.*, 2009). The expression of *VvMYB5a* and *VvMYB5b* genes was previously detected by Deluc *et al.* in many grapevine organs. In berry they are both expressed before veraison, while only *VvMYB5b* was also expressed in the skin tissues during the ripening (Deluc *et al.*, 2006; Deluc *et al.*, 2008).

Given the low efficiency of transformation and the long time of regeneration of grapevine, to date functional analysis of VvMYB5a and VvMYB5b was mainly performed in heterologous systems. Constitutive expression in tobacco strongly impacted the metabolism of anthocyanins, flavonols, tannins and lignins in reproductive organs (Deluc *et al.*, 2006; Deluc *et al.*, 2008). In grape cells, they both activated the promoters of early structural genes of the flavonoid pathway (Deluc *et al.*, 2008). Considering these results, it was hypothesized that VvMYB5a is particularly involved in the control of PA synthesis in skin and seed tissues before veraison, whereas VvMYB5b may participate in the regulation of both proanthocyanidin and anthocyanin biosynthesis throughout berry development. Recent experiments revealed, however, that VvMYB5b is probably implicated in the regulation of other metabolic processes, given by the expression in tomato affected not only the secondary metabolism, but also wax deposition, cell size and shape (Mahjoub *et al.*, 2009). In chapter 2, we showed that when expressed in petunia flowers, VvMYB5a and VvMYB5b are able to regulate vacuolar acidification and they may be also involved in the vesicle transport.

Despite the expression in heterologous systems can be extremily useful in defining the gene function, the results obtained are sometimes controversial and may not reflect the real role of VvMYB5a and VvMYB5b in grapevine, probably because in a host species they mimic the functions of the orthologous regulator. It is therefore necessary carrying on the functional analyses directly in the native species. Here we described the characterization of VvMYB5a and VvMYB5b in *Vitis vinifera* with two different approaches: ectopic expression in hairy roots and stable transformation of embryogenic callus.

MATERIALS AND METHODS

Plasmid constructs

For the overexpression of *VvMYB5a* and *VvMYB5b* in hairy roots of *Vitis vinifera* ’Corvina’, the pENTR/D-TOPO (Invitrogen) vectors used were described in chapter 2. The coding sequences were transferred into the binary overexpression vector pH7GW2D (Laboratory of Plant Systems Biology, PSB; Ghent University, Belgium) by site-specific recombination. The constructs were then inserted into *Agrobacterium rhizogenes* strain A4 by triparental mating and the transformed strain was then used to transform *Vitis vinifera* ‘Corvina’.

For the overexpression of *VvMYB5a* and *VvMYB5b* in *Vitis vinifera* ‘Shiraz’, the vectors pART7 used (Gleave, 1992) were previously engineered for the presence of the coding regions as described by Deluc *et al.* (2008). Each expression cassette, containing the respective ORF under the control of the CaMV *35S* promoter, was isolated as *NotI* fragment, ligated to the *NotI* site of the binary vector pART27-4a (Gleave, 1992), and transferred into *Agrobacterium tumefaciens* EHA105 by electroporation. For *VvMYB5a* / *VvMYB5b* RNA silencing, the vector pDHA10 used (Shang *et al.*, 2007) was previously engineered in CSIRO Plant Industry. It incorporates 303 bp specific sequences of both *VvMYB5a* and *VvMYB5b* cds one downstream the other in sense and antisense orientation to form the hairpin structure that triggers RNA silencing. Sequently, the construct was ligated as a *NotI* fragment to the binary vector pART27-4a (Gleave, 1992) modified by Walker *et al.* to contain a constitutively-expressed *GFP* gene (unpublished). This vector was transferred to the Agrobacterium strain EHA105 by electroporation.

Genetic transformation procedures

For hairy roots formation, we collected branches of *Vitis vinifera* ‘Corvina’ plants grown in glasshouse. The samples were cut into 3 cm pieces and sterilized. The tip of each cutting was inoculated with the *Agrobacterium rhizogenes* A4 strain previously grown on YMB medium and then transferred into semi/solid HB medium (Hoos and Blaich, 1988). The roots grown on the top of the cuttings were cultured on solid MS medium (Murashige and Skoog, mod. 1B, Duchefa) supplemented with with 100 µg/ml amoxicillin and 100 µg/ml cefotaxime. The hairy root lines were subcultured every week on the same medium.

For the genetic transformation of *Vitis vinifera* ‘Shiraz’, embyogenic callus was developed from anthers collected during the 2008 and 2009 seasons from Coombe Vineyard, University of Adelaide, Urrbrae, South Australia. Once initiated, the callus was maintained on C1 medium in dark at 28°C and subcultured every 4 weeks. Well-developed material was selected for the transformation and maintained into GS1CA media for 14 days. For inoculation with *Agrobacterium tumefaciens* strain EHA105, the embryogenic callus was collected in a Petri dish and submerged in 3 ml of bacterial suspension for 10 minutes. Bacterial suspension was withdrawn using a transfer pipette and any excess moisture was removed by blotting with sterilized Whatman 3MM filter paper. The agro-infiltrated callus was transferred to a new Petri dish containing 3 pieces of filter paper saturated with liquid modified GS1CA and incubated in the dark at 22°C. After 48 hours, the embryogenic callus was washed in liquid culture media with timentin (1000 µg /ml) and placed onto GS1CA medium for 9-10 days in the dark at 28°C. To select the transformed material, the callus was then moved into GS1CA supplemented with kanamycin 150 µg/ml; four weeks after the transformation they were subcultured into NN media with the selection antibiotic. Continuing to subculture the material every 4 weeks allowed the germination of GFP positive embryos which were selected at the microscope and collected on MS/2 with 5µM BAP. When primary shoots emerged, the embryos were transferred firstly in the same media without hormones to help the shoot elongation. Subsequently, plantlets were cut off and transferred into Magenta vessels containing rooting medium and cultured under the same conditions to allow further plant development. Vigorous transgenic plants with well-developed leaves and roots were then transplanted into 7-cm plastic pots containing grape soil mix and acclimated in the greenhouse under the shade for about 3 weeks before transfer to the light.

Genomic DNA extraction and Southern Blot

Genomic DNA was extracted from 1 g of leaves (between 1 and 3 cm in length) by the method of Thomas and Scott (1993). For southern blot analysis, genomic DNA (2 µg) was restricted overnight with 20 units of the restriction enzyme *EcoRV* at 37° C. Digests were electrophoresed overnight on 0.7% agarose gels in 1 x TBE buffer. Southern blots were performed using the salt transfer method to Genescreen Plus (Perkin Elmer Life Sciences) and hybridised overnight at 65°C with DNA probes labelled using Rediprime TM (Amersham Biosciences) with ^{32}P-dATP. The filters were washed in 2 x SSC + 1% SDS at 65° C for 10 min and then 0.1 x SSC + 1% SDS at 60° C for 10 min. Filters were exposed to Biomax MS film (Kodak) using Biomax (Kodak) intensifying screens at –8° C for 5 h to 2 days.

Transcriptomic analyses

For gene expression analyses of transgenic grapevines cv ‘Shiraz’, total RNA was isolated using the Spectrum Plant Total RNA kit (Sigma-Aldrich) according to the manufacturer’s instructions. The eluted RNA was further purified with Lithium Chloride 7,5 M.

First-strand cDNA synthesis was performed with SuperScript III First-Strand Synthesis System (Invitrogen). Transcript levels of *VvMYB5a* and *VvMYB5B* were measured by real time RT-PCR, using SYBR green method (ABgene House) on a Rotor-Gene 2000 (version 4.2) real time cycler (Corbett Research). The thermal cycling conditions were 95°C for 15 min followed by 95°C for 30 s, 58° C for 25 s, and 72° C for 25 s for 35 cycles, followed by a melt cycle from 50° C to 96° C. The list of gene-specific primers designed close to 3’UTR is reported in Table 1. All samples measured in triplicate were normalized to *VvUbiquitin1* gene. The difference between the cycle threshold (Ct) of the target gene and the Ct of Ubiquitin, $\Delta Ct = Ct_{Target} - Ct_{Ubiquitin}$, was used to obtain the normalized expression of the target gene, which corresponds to 22DCt. The Rotor Gene 2000 software (Corbett Research) and the Q-Gene software (Muller *et al.*, 2002) were used to calculate the mean normalized expression of the genes.

Table 1. List of primer used for expression analyses by Real time RT-PCR.

GENE	PRIMER NAME	SEQUENCE 5'-3'
VvUBIQ1	VvUBIQUITIN FOR	GTGGTATTATTGAGCCATCCTT
	VvUBIQUITIN REV	AACCTCCAATCCAGTCATCTAC
VvMYB5a	VvMYB5A FOR	ACATCGGCTCCTTTAGTCCA
	VvMYB5A REV	GCTAATACAACATCAAAACACAA
VvMYB5b	VvMYB5b FOR	GGTGTTCTTTAATTTGGCTTCA
	VvMYB5b REV	CACAACAACACAACCACATACA
VvMYBF1	VvMYB12 FOR	TGGAGGTTGAGGGGTTGTG
	VvMYB12 REV	AAGTTGGGGAAGAGCAGGAG
VvMYBPA2	VvMYBPA2 FOR	GACATTGGCGACCTCCTTAC
	VvMYBPA2 REV	CGAGAAAATCGGAACAAGGA
VvANS	VvBAN FOR	CAATACCAGTGTTCCTGAGC
	VvBAN REV	AAACTGAACCCCTCTTTCAC
VvLAR1	VvLAR FOR	ACTCTGCAATTGCCAACAC
	VvLAR REV	CCCATGTTACAACAAGGAGTAG
VvUFGT	VvUFGT FOR	CAACACATTGTGGATGGAACTCAT
	VvUFGT REV	ACCTTCAATTCTCACTCCAATCTC
VvFLS1	VvFLS FOR	CAGGGCTTGCAGGTTTTTAG
	VvFLS REV	GGGTCTTCTCCTTGCACG

Microarray analysis

Total RNA for microarray analysis was isolated from ~100 mg of the ground leaves tissue using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich). RNA quality and quantity were determined using a Nanodrop 2000 instrument (Thermo Scientific) and a Bioanalyzer Chip RNA 7500 series II (Agilent). The cDNA synthesis, labeling, hybridization and washing reactions were performed according to the NimbleGen Arrays User's Guide (V 3.2). Each hybridization was carried out on a NimbleGen microarray 090818 Vitis exp HX12 (Roche, NimbleGen Inc.), representing 29,549 predicted genes on the basis of the 12X grapevine V1 gene prediction version (http://srs.ebi.ac.uk/). The chip probe design is available at the following URL: http://ddlab.sci.univr.it/FunctionalGenomics/. The microarray was scanned using a ScanArray 4000XL (Perkin-Elmer) at 532 nm (Cy-3 absorption peak) and GenePix

Pro7 software (Molecular Devices) according to the manufacturer’s instructions. Images were analyzed using NimbleScan v2.5 software (Roche), which produces Pair Files containing the raw signal intensity data for each probe and Calls Files with normalized expression data derived from the average of the intensities of the four probes for each gene. The normalized gene expression data were finally converted in log2 values to process data. A Pearson Correlation analysis was carried out to evaluate the robustness of the three biological replicates in each sample. A gene was considered to be expressed if the normalized expression value was higher than the value obtained by averaging the fluorescence of negative control present on the chip for at least two of the three biological replicates. A Significance Analysis of Microarrays (SAM) was implemented using TMeV software (http://www.tm4.org/mev), with a false discovery rate of 0,2% for *35S::VvMYB5a* and *35S::VvMYB5b* and 1,2% for *VvMYB5a / VvMYB5b* silencing. Cluster analysis was performed by the k-means method with Pearson's correlation distance (TMeV) referring EV and H gene expression to BV.

Histology and histochemical staining of leaf sections

For histological studies, sections including the principal veins were obtained from wild type and transgenic leaves. Each sample was successively fixed in FAA solution (formaldeyde, acetic acid and ethanol), dehydrated in several ethanol dilutions and embedded in Spurr’s resin (Spurr, 1969). Semithin sections, 2.5 mm thick, were stained by 0.05% toluidine blue in sodium tetraborate containing water. Lignin-containing cell walls are heavily blue stained. For the histochemical staining of lignin monomers, the fresh leaf tissues were bleached in 100% ethanol for 5 days, incubated in a solution of 1% (w/v) phloroglucinol – (25%) w/v HCl (Waldron KW, 1997) and then washed three times with water. CTs were visualized according to Porter (1989) by incubating tissue slices in a mixture of ethanol:6 M HCl (1:1, v/v) containing 0.1% (w/v) DMACA (Sigma-Aldrich) for 3 to 6 min, then washing three times with water.

All the samples were then observed with a Zeiss Axiophot microscope and digitized pictures were obtained with a Spot camera (Diagnostic Instruments).

RESULTS

Ectopic expression of *VvMYB5a* into grapevine hairy roots

In order to establish the function of VvMYB5a and VvMYB5b, the full-length cDNAs driven by the *35S* promoter were separately introduced into hairy roots of *Vitis vinifera* ‘Corvina’. Hairy roots were screened by PCR for the presence of the kanamacyn resistence gene from the pK2GW7D backbone, yielding three positive independent transgenic lines from the transformation with *35S::VvMYB5a* construct. No hairy roots were obtained by the ectopic expression of *VvMYB5b*. Both *35S::VvMYB5a* and wild type hairy roots presented a thick diameter (2–3 mm), an active growth and no secondary branching. They appeared white-yellow and depending on growth rate, they presented pink-red pigmentation in the basal portion.

In order to discover the whole set of downstream genes that VvMYB5a regulates, a transcriptomic analysis using a 90 K *Vitis vinifera* oligoarray was conducted on the three biological replicates in comparison to three untransformed lines. Considering a 3-fold cut off, 767 genes resulted to be up-regulated, while 264 were down-regulated, suggesting that VvMYB5a is involved in the regulation of numerous processes such as phenylpropanoid pathway, cell wall metabolism, hormone-signalling and photosynthesis. Among the up-regulated genes, of particular interest are those related to the general pathway of the flavonoid synthesis (Table 2), such as chalcone synthase and flavonoid 3-monooxygenase. The flavonol structural gene *VvFLS* as well as the proanthocyanin genes *VvLAR1* and *VvANR* were identified, revealing VvMYB5a involvement in the regulation of these specific branches of the pathway. *VvMYB5a* overexpression caused the induction of genes related to the phenylpropanoid pathway as cinnamoyl-CoA reductase, sinapyl alcohol dehydrogenase and cinnamyl alcohol dehydrogenase, emerged also in transcriptomic experiments in petunia plants (Chapter 2).

As demonstrated in chapter 2, VvMYB5a and VvMYB5b can regulate the vacuolar pH in petunia plants. For this reason, we also looked into those up-regulated genes that may play an important role in acidification of cellular compartments. Three genes with high homology to the plasma membrane H+-ATPases *AHA2*, *AHA4* and *AHA10* of Arabidopsis were recognized. We also observed the induction of two cysteine proteinases, that resemble the modulation of the putative cysteine proteinase PhCAC16.5 by PhPH4 in *Petunia hybrida*. The homolog of Arabidopsis *TRANSPARENT TESTA GLABRA 2 (TTG2)* and petunia *PH3*, that we identified as *VvWRKY19* in chapter 4, was up-regulated. These microarray data suggest that also in grapevine a similar acidification pathway is controlled by regulators like VvMYB5a. In addition, we identified two sequences homologous to the Arabidopsis regulators *WEREWOLF* and *CAPRICE* that compete to define cell fates in the Arabidopsis root epidermis (Song *et al.*, 2011).

Taken together, these data confirmed VvMYB5a as regulator of the general flavonoid pathway and provided evidences of its involvement in the control of flavonol and proanthocyanidin structural genes like flavonol synthase and anthocyanidin reductase. However, given the high number of modulated genes, we also proposed that VvMYB5a regulates many other metabolic and developmental processes.

Table 2. Up-regulated genes belonging to the flavonoid metabolism in *35S::VvMYB5a* hairy roots.

SEQ_ID	GENE DESCRIPTION	FC
VIT_14s0068g00930	Chalcone synthase 1	36.7
VIT_09s0002g01090	Flavonoid 3'-monooxygenase	12.8
VIT_05s0136g00260	Chalcone synthase 3	9.9
VIT_02s0025g04720	Leucoanthocyanidin dioxygenase	8.9
VIT_00s0361g00040	Anthocyanidin reductase (BAN)	7.4
VIT_18s0001g09400	Cytochrome b5 (DIF-F)	6.2
VIT_01s0011g02960	Leucoanthocyanidin reductase 1	5.4
VIT_17s0000g07210	Flavonoid 3'-monooxygenase (F3'H)	4.8
VIT_04s0023g03370	Flavanone-3-hydroxylase (F3H)	4.7
VIT_16s0022g01540	Flavonoid 3',5'-hydroxylase	4.5
VIT_18s0001g03430	Flavonol synthase	3.9
VIT_13s0067g02870	Chalcone--flavonone isomerase	3.7
VIT_16s0022g01500	Flavonoid 3'-monooxygenase	3.5
VIT_18s0001g14310	Flavanone 3-hydroxylase (F3H)	3.3

Characterization of *VvMYB5a / VvMYB5b* silenced plants

A second approach used to examine the roles of VvMYB5a and VvMYB5b in the native species was the stable transformation of embryogenic callus of *V. Vinifera* 'Shiraz' with *Agrobacterium tumefaciens.* Two separate experiments were performed in order to silence and overexpress *VvMYB5a* and *VvMYB5b*.

Because of their overlapping functions, two specific sequences of *VvMYB5a* and *VvMYB5b* were cloned one downstream the other in the vector pDAH10 in order to silence the expression of both genes in the same event of transformation and avoid compensation effects. The presence of the *GFP* sequence under the control of Arabidopsis *Ubiquitin10* promoter and terminator in the vector allowed the additional selection of the transformed embryogenic material during the development of the embryos. We firstly selected 60 transformed plantlets generated from 270 GFP positive embryos. Among them, six lines were chosen for the lowest expression of *VvMYB5a* and *VvMYB5b* (Figure 1).

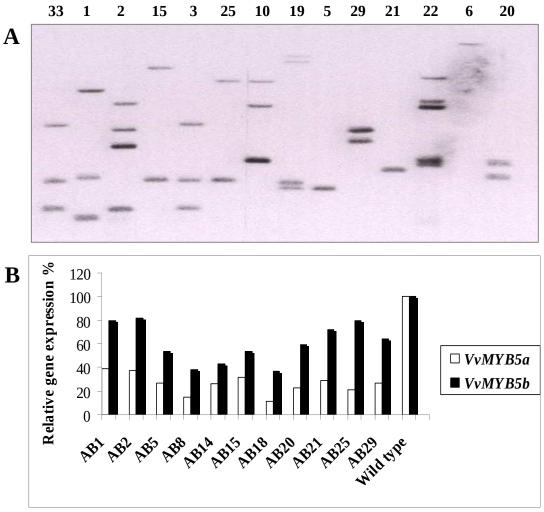

Figure 1. Selection of the most silenced lines for *VvMYB5a* and *VvMYB5b* expression.

A) Example of southern blot analysis performed to select the transgenic lines with a single copy of the transgene.

B) Real time RT-PCR analysis of *VvMYB5a* and *VvMYB5b* expression of leaves of silenced grapevines. The selected lines are AB5, AB8, AB18, AB20, AB21 and AB25.

Four of these plants contained one insertion of the transgene (AB5, AB20, AB21 and AB25) while two plants were characterized by two copies (AB8, AB18). The severity of the symptoms observed in the transgenic plants correlates with the copy number of the transgene and the level of silencing expression.

VvMYB5a / VvMYB5b silencing plants showed stunted growth with a reduce dimension in comparison to the wild type plant (Figure 2A). The leaves, which appeared thick and curly, were characterized by anthocyanin accumulation in the epidermis and by thicker veins that seemed lignified (Figure 2C).

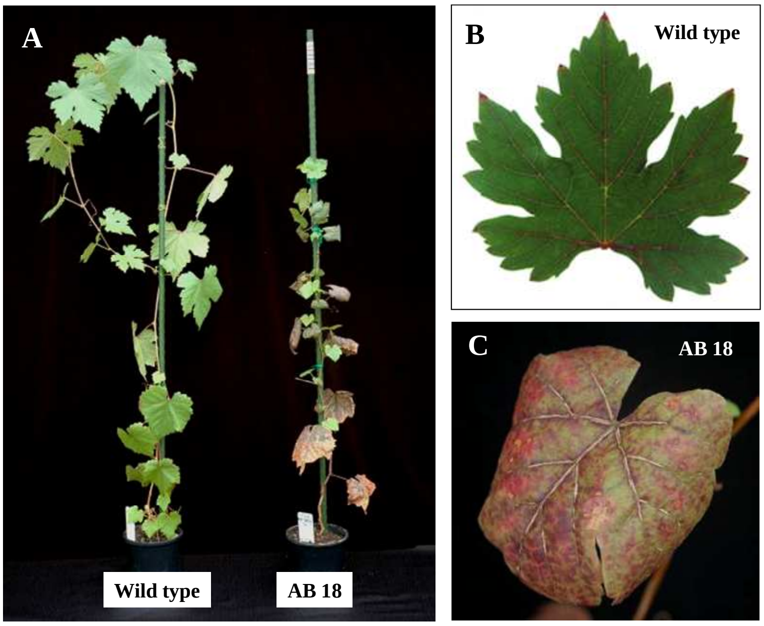

Figure 2. Phenotypes of grapevine silenced for *VvMYB5a* and *VvMYB5b* (AB18) in comparison to the wild type. Transgenic plants and the respective control were maintained in the same conditions in the glasshouse.

A) *VvMYB5a* / *VvMYB5b* silenced plants presented stunted growth and reduced dimension when compared to the wild type.

B-C) Leaves of wild type (**B**) and AB18 transgenic plants (**C**) that show an increased pigmentation in epidermis and vein lignification.

VvMYb5a and VvMYB5b as regulators of PA and flavonols synthesis

The transcriptomic analysis on hairy roots, above described, revealed that at least VvMYB5a is able to induce the expression of the structural genes *VvLAR1* and *VvANR* for PA synthesis and *VvFLS* of flavonol branch. Real time RT-PCRs of figures 3A, 3B and 3C show the down-regulation of *VvLAR1*, *VvANR* and *VvFLS* expression in *VvMYB5a / VvMYB5b* silencing leaves in comparison to the control. Following these indications, VvMYB5a and VvMYB5b could be specific regulators of the flavonoid branches for proanthocyanidin and flavonol synthesis. It is therefore plausible that *VvMYB5a / VvMYB5b* silencing causes redirection of the intermediate compounds of the flavonoid pathway preferentially to the anthocyanin production rathern than PA and flavonol synthesis, explaining the anthocyanin accumulation in leaf epidermis of the transgenic plants. In line with this hypothesis, the expression of *VvUFGT* gene specific for anthocyanin branch, appeared slightly up-regulated in the transgenic plants (Figure 3D). Alternatively, the appearance of anthocyanins could be interpreted as a plant response to a stress condition (Chalker-Scott, 1999) representing a secondary effect of the silencing. To have more detailed information about this regulative mechanism, we verified the expression of *VvMYBPA2* and *VvMYBF1* genes responsible for proanthocyanidin and flavonol synthesis (Czemmel *et al.*, 2009; Terrier *et al.*, 2009), respectively. *VvMYBPA1* gene was not considered in this analysis because its expression was proven only in grape berries and seeds but not in leaves (Bogs *et al.*, 2007). Data obtained revealed that also *VvMYBPA2* and *VvMYBF1* expression was down-regulated in transgenic plants (Figure 3E and F), possibly explaining the down-regulation of PA and flavonol structural genes, respectively. However, experiments of promoter activation in grape cells could not demonstrate that VvMYB5a and VvMYB5b directly activate *VvMYBPA2* and *VvMYBF1* expression (data not shown) possibly because of the presence of other intermediate regulators in the regulatory network. Another explanation is that *VvMYB5a / VvMYB5b* silencing may perturb other metabolic processes inducing indirect changes in gene expression for *VvMYBPA2* and *VvMYBF1*.

Taken together, all these findings suggest that VvMYB5a and VvMYB5b are directly or indirectly involved in the regulation of flavonol and proanthocyanidin synthesis in leaves.

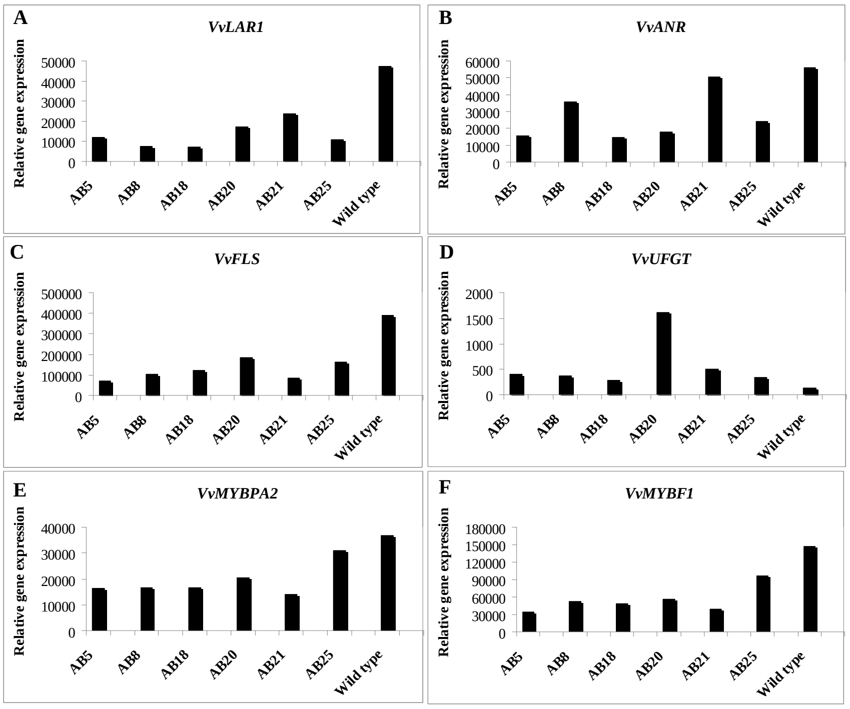

Figure 3. Expression analyses of genes related to flavonoid synthesis in *VvMYB5a / VvMYB5b* silenced grapevines by real time RT-PCR. Data, relative to expression of *VvUbiquitin1* gene, are the mean of two replicates. The analysis was conducted for the proanthocyanidin (**A-B**), flavonol (**C**) and anthocyanin strtuctural genes (**D**). **E-F** represent the real time RT-PCRs performed for the expression of the each specific regulator of the flavonoid branches.
Abbreviations are as follows: VvLAR1, leucoanthocyanidin reductase; VvANR, anthocyanidin reductase; VvFLS, flavonol synthase; VvUFGT, UDP-Glc:flavonoid 3-O-glucosyltransferase.

Different organization of leaf parenchima and modification of lignin deposition in *VvMYB5a / VvMYB5b* silenced leaves

For a deep analysis of the phenotypic effects observed on the veins, leaf sections of transgenic and wild type plants were stained with phloroglucinol-HCL. A red coloration, linked to the presence of monomers of lignin, was observed in the veins of transgenic leaves (Figure 4D), but not in control line (Figure 4B). This atypical deposition of lignin could explain the thickness of the tissue as well as the presence of secondary structures grown on the principal vein of the transgenic plants (Figure 4C). The involvement of VvMYB5a in lignin metabolic pathway was already deduced by previous experiments in tobacco (Deluc *et al.*, 2006), by complementation analyses in petunia mutants (Chapter 2) and ectopic expression in grape hairy roots described in this thesis. Here we demonstrated that *VvMYB5a / VvMYB5b* silencing led to altered lignin deposition. However, the causes of this phenotypic effects remain to be elucidated. It is still unknown how these transcription factors influence the pathway. They may directly regulate the enzymatic steps of lignin synthesis. On the other side, the reduce activation of flavonoid structural genes caused by *VvMYB5a / VvMYB5b* silencing could result in redirection of phenylpropanoid pathway to lignin synthesis.

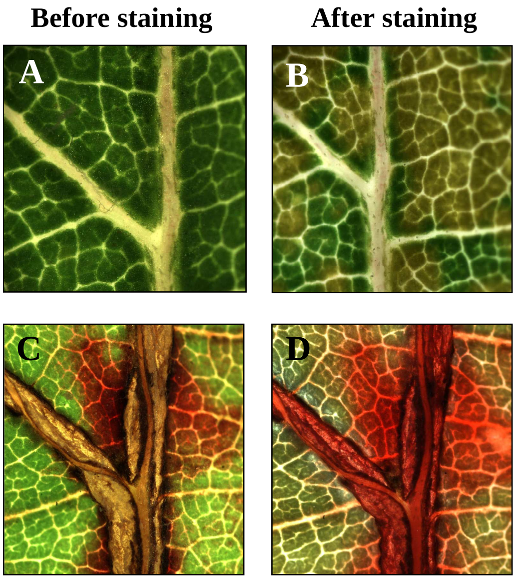

Figure 4. Principal veins and epidermis of wild type (**A-B**) and transgenic leaves (**C-D**). Phloroglucinol-HCl staining of leaf section from transgenic plant AB18 (**D**) shows monolignols accumulation when compared to control line (**B**).

Another phenotypic modification affected the leaf veins of the silenced plants, as shown in sections of figure 5. The parenchymal cells presented abnormal cell dimension and shape in the transgenic plants (Figure 5B) in comparison to the wild type (Figure 5A). The outer layer of epidermal cells was not well distinguishable and seemed to be replaced by the parenchyma cells. Moreover, we observed different cell organization in the mesophyll of *VvMYB5a / VvMYB5b* silencing leaves. The sections of figure 5C and 5D revealed that the palisade mesophyll was not present and it was completely substituted by the spongy mesophyll.

In conclusion, the silencing of *VvMYB5a / VvMYB5b* expression does not affect only the flavonoid pathway, but also many other phenotypic traits. We therefore concluded that VvMYB5a and VvMYB5b are involved in the regulation of multiple developmental processes.

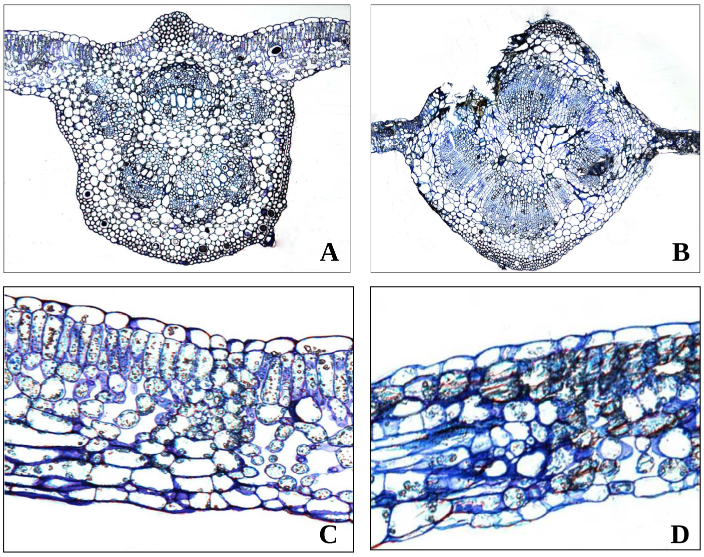

Figure 5. Cross sections of veins from control (**A**) and *VvMYB5a* / *VvMYB5b* silenced plant AB18 (**B**). Magnifications representing leaf epidermis and mesophyll of control (**C**) and *VvMYB5a* / *VvMYB5b* silenced plant AB18 (**D**).

Microarray analysis on *VvMYB5a / VvMYB5b* silenced plants

In order to characterize putative changes at transcriptomic level caused by *VvMYB5a / VvMYB5b* silencing, a microarray analysis was performed on leaf RNA of transgenic and wild plants. As biological replicates, we collected three young leaves from the same plant at the same stage of development when the phenotype was not visible yet. A multiclass comparison analysis was carried out using Significance Analysis of Microarray (SAM) with a false discovery rate (FDR) of 1,2 % (TMev 4.3). Considering a fold change > |3|, we identified 134 down-regulated and 66 up-regulated genes (Supplementary table 2), which were annotated using V1 version of the 12X draft annotation of the grapevine genome (http://genomes.cribi.unipd.it/DATA/) and distributed into 18 Gene Ontology (GO) functional categories (Figure 6).

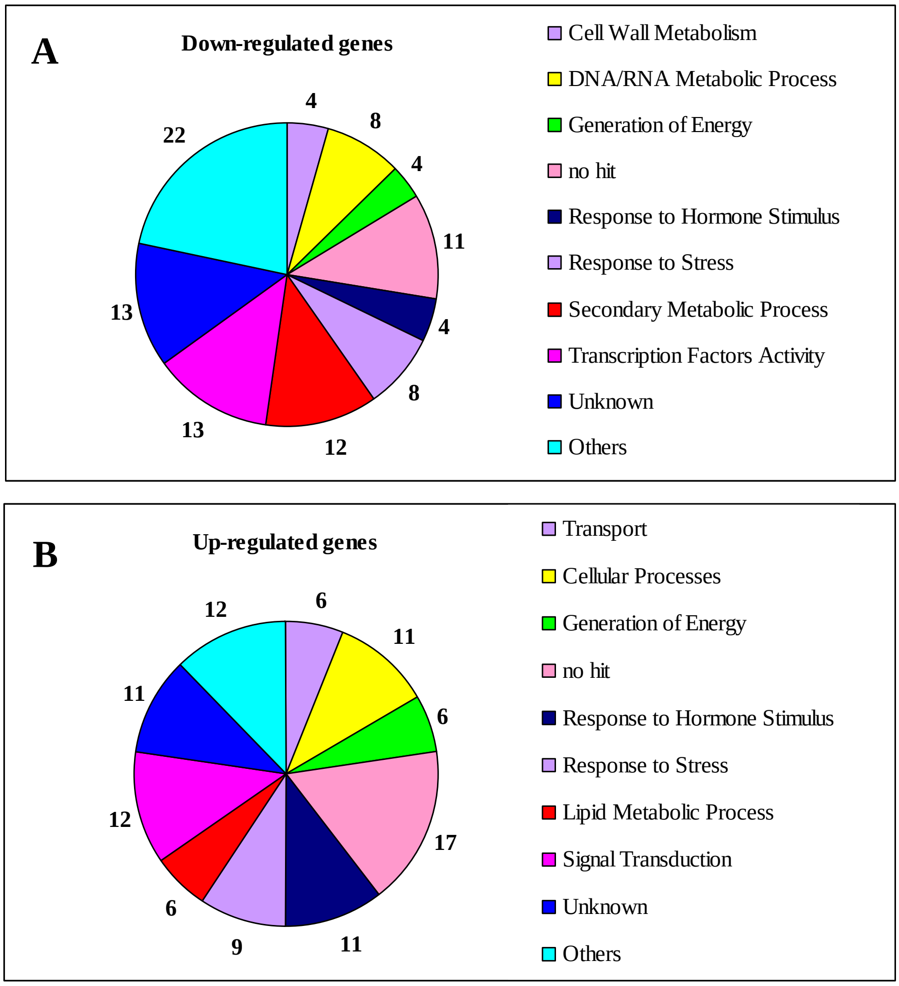

Figure 6. Distribution (%) of down-regulated (**A**) and up-regulated genes (**B**) in *VvMYB5a / VvMYB5b* silenced plants in to 18 Gene Ontology (GO) functional categories.

Table 3. The 50 most repressed genes in *VvMYB5a / VvMYB5b* silenced leaves compared to the untransformed line.

SEQ_ID	GENE DESCRIPTION	FC
VIT_17s0119g00280	alpha-amylase/subtilisin inhibitor	-21.1
VIT_18s0075g00270	no hit	-17.0
VIT_18s0086g00410	auxin-binding protein ABP19	-16.7
VIT_05s0077g01150	Beta-1.3-glucanase	-15.7
VIT_18s0001g00740	ornithine decarboxylase	-15.5
VIT_12s0028g02990	fasciclin arabinogalactan-protein (FLA13)	-14.6
VIT_14s0006g03210	unknown	-13.0
VIT_05s0094g00340	Chitinase class IV	-12.0
VIT_07s0130g00030	proteinase inhibitor se60	-10.6
VIT_16s0100g01030	stilbene synthase [Vitis quinquangularis]	-10.5
VIT_12s0034g01900	globulin-like protein	-10.1
VIT_16s0100g00760	Stilbene synthase 3	-9.7
VIT_12s0034g01920	no hit	-9.6
VIT_17s0000g08460	carbonic anhydrase. chloroplast precursor	-9.3
VIT_19s0027g01880	amino acid transport protein	-8.3
VIT_15s0048g00510	pectinesterase family	-8.1
VIT_08s0105g00380	Leucoanthocyanidin dioxygenase	-7.7
VIT_12s0059g01410	no hit	-7.7
VIT_12s0034g01970	cupin	-6.8
VIT_03s0088g00050	serine carboxypeptitase 1	-6.8
VIT_18s0164g00050	no hit	-6.8
VIT_18s0001g13130	BEL1-LIKE HOMEODOMAIN 10	-6.8
VIT_03s0091g00390	snakin-1	-6.7
VIT_02s0025g00750	pinoresinol forming dirigent protein	-6.6
VIT_07s0005g05930	auxin-binding protein ABP19	-6.5
VIT_18s0075g00460	TIR-NBS-LRR disease resistance	-6.5
VIT_05s0020g02200	inosine-uridine preferring nucleoside hydrolase	-6.3
VIT_16s0100g00960	stilbene synthase [Vitis pseudoreticulata]	-6.3
VIT_16s0100g00940	stilbene synthase 3 [Vitis sp. cv. 'Norton']	-6.1
VIT_07s0031g00770	Unknown protein	-5.8
VIT_03s0088g00110	serine carboxypeptidase SCPL17	-5.8
VIT_00s0228g00060	unknown	-5.8
VIT_18s0001g06140	Phosphate-induced protein 1	-5.6
VIT_00s0480g00040	polyphenol oxidase II. chloroplast precursor	-5.6
VIT_15s0046g00520	wax synthase	-5.5
VIT_02s0025g04330	Thaumatin VVTL1 [Vitis vinifera]	-5.3
VIT_09s0002g01320	germin-like protein	-5.3
VIT_19s0027g01870	Amino acid permease	-5.1
VIT_11s0016g05540	dicyanin	-5.1
VIT_02s0033g01390	no hit	-4.9
VIT_08s0040g01490	unknown	-4.7
VIT_13s0067g00840	TTN10 (TITAN 10)	-4.7
VIT_10s0042g00840	stilbene synthase [Vitis pseudoreticulata]	-4.6
VIT_17s0000g08450	carbonic anhydrase chloroplast	-4.6
VIT_18s0075g00440	TIR-NBS-LRR disease resistance	-4.6
VIT_17s0119g00160	trypsin and protease inhibitor Kunitz family	-4.4
VIT_08s0007g08750	heat shock transcription factor B3	-4.4
VIT_03s0091g00650	no hit	-4.3
VIT_02s0025g00760	pinoresinol forming dirigent protein	-4.3

As putative targets of VvMYB5a and VvMYB5b, we firstly focused our attention on the down-regulated genes, which appear involved in different processes (Table 3; Supplementary table 2). Among these, we identified genes belonging to the “Secondary metabolic processes” category, such as five stylbene synthases, probably associated to stress response (Wang *et al.*, 2010). Other phenylpropanoid genes identified were related to the synthesis of the lignan pinoresinol, as the pinoresinol forming dirigent protein and the pinoresinol-lariciresinol reductase (Suzuki S., 2007). A leucoanthocyanidin dioxygenase and an anthocyanidin 3-O-glucosyltransferase were the only genes linked to the flavonoid metabolism. Many auxin binding proteins which were grouped in the category “Other” appeared to be down-regulated in *VvMYB5a / VvMYB5b* silenced plants, possibly explaining the smaller dimension, the stunted growth and also the abnormal leaf mesophyll of the transgenic plants.

Many genes were related to the “Transcription factor activity”, suggesting that VvMYB5a and VvMYB5b could control downstream pathways through the modulation of the expression of intermediate regulators. Among these, we identified a sequence with high similarity to *WEREWOLF* that defines cell fates in Arabidopsis root epidermis (Lee and Schiefelbein, 1999; Song *et al.*, 2011). A homolog of Arabidopsis *TRIPTYCHON*, a R3 MYB factor known to mediate lateral inhibition during trichome and root hair patterning (Schellmann *et al.*, 2002), was also included in this category. Information regarding VvMYB5a and VvMYB5b role in the control of putative negative regulators was already inferred by microarray analyses on petunia transgenic plants (Chapter 2) and *VvMYB5a* overexpressing grape hairy roots described in this chapter. However, the role of these negative regulators as well as their competition with other MYB factors has still to be verified in grapevine.

We identified genes associated to the “DNA/RNA Metabolic Process”, as DNA polymerase δ, helicase and spindle checkpoint protein involved in the DNA replication. The “Unknown” and “no hit” categories were represented with a percentage of 13 and 11, respectively.

Among the up-regulated genes, we noted that many categories are almost equally represented (Figure 6B), again indicating that many aspects of the cell metabolism could be influenced by the simoultaneus silencing of *VvMYB5a* and *VvMYB5b* expression, as suggested by the high percentage of genes related to the signal transduction. It is worth mentioning the up-regulation of some ethylene-responsive transcription factors because ethylene has been shown to have a promotive effect on light-induced anthocyanin synthesis, as suggested by Craker et al (1973). However, neither genes related to anthocyanin or lignin production, that could explain the phenotypic features of the transgenic plants, were identified in this analysis. Taken together, all these data suggest that in leaves the silencing of *VvMYB5a* and *VvMYB5b* expression caused the modulation of genes involved in different metabolic processes. It remains to clarify if this is due to a direct regulation of the gene expression or to stress conditions of the silenced plants.

Characterization of *VvMYB5a* and *VvMYB5b* overexpressing plants

To further investigate the functions of VvMYB5a and VvMYB5b and to highlight putative functional differences between the two factors, *Vitis vinifera* ‘Shiraz’ was also transformed to overexpress them independently.

The transformation events yielded 180 and 260 transformed embryos for VvMYB5b and VvMYB5a, respectively. For each construct, we firstly selected 40 PCR positive plants and among these, 6 lines for a single insertion and the highest expression of the transgene and for the presence of the phenotype (Figure 7).

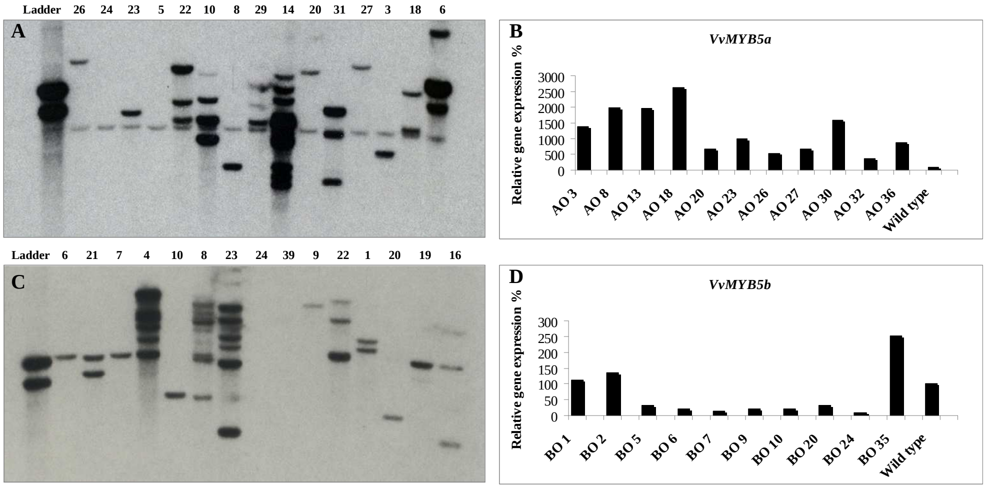

Figure 7. Selection of lines with highest expression and single copy of *VvMYB5a* and *VvMYB5b* transgenes.

A-C) Examples of southern blot analyses to select the transformants with a single copy of *35S::VvMYB5a* and *35S::VvMYB5b* transgenes, respectively.

B-D) Real time RT-PCR analyses of *VvMYB5a* and *VvMYB5b* expression in leaves of silenced grapevines. The selected lines are AO 3, AO 8, AO 13, AO 18, AO 23 and AO 30 for *35S::VvMYB5a*. and BO 1, BO 2, BO 6, BO 9, BO 10 and BO 35 for *35S::VvMYB5b*.

VvMYB5a overexpressing plants presented growth rate and dimension similar to the wild type line. Modifications were visible on the leaf epidermis with necrotic regions close to the edges of the leaf (Figure 8B).

The same phenotypic characteristic is evident also for *VvMYB5b* overexpressing leaves, but the necrosis is localized in the inter-vein regions of the epidermis as shown in figure 8C.

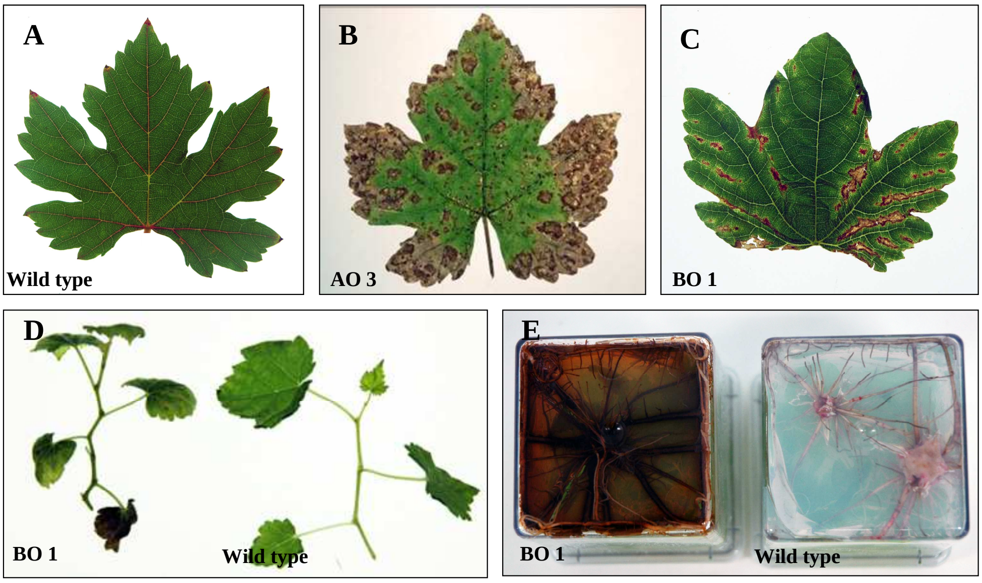

Figure 8. Phenotypes of *VvMYB5a* and *VvMYB5b* overexpressing plants in comparison to the wild type. Transgenic plants and the respective control were maintained in the same conditions in the glasshouse.
A-B-C) Leaves of wild type (**A**), *35S::VvMYB5a* (**B**) and *35S::VvMYB5b* (**C**) leaves.
D) Comparison between *VvMYB5b* overexpressing (BO1) and the control plants.
E) Roots of *in vitro* 35S::*VvMYB5b* and control plants. The overexpression of *VvMYB5b* caused production of brown compounds released into the medium.

Accumulation of condensed tannins in leaves was detected using dimethylaminocinnamaldehyde (DMACA) staining (Xie *et al.*, 2003). A blue staining, linked to the presence of PA, was observed in the epidermal cell layers of *VvMYB5b* overexpressing leaves (Figure 9A) when compared to the control (Figure 9B). The presence of PA in *35S::VvMYB5a* plants has still to be verified.

The overexpression of *VvMYB5b* affected also the plant dimension, which was characterized by short internodes and small curly leaves (Figure 8D). Similar effects were described for tomato transgenic plants, where *VvMYB5b* overexpression caused pleiotropic changes including dwarfism and modified leaf structure (Mahjoub *et al.*, 2009). In addition, the *in vitro* plantlets presented brown roots that released dark compounds in the medium (Figure 8E). Although we hypothesized an abnormal accumulation of proanthocyanidins in roots and leaves, further analyses are required for a deeper phenotypic characterization of the transgenic plants.

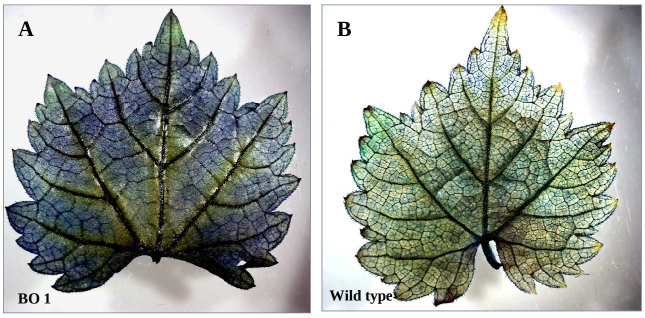

Figure 9. DMACA staining of leaves from *VvMYB5b* transgenic plant BO1 (**A**) shows condensed tannin accumulation when compared to control line (**B**).

Microarray analysis on *VvMYB5a* and *VvMYB5b* overexpressing plants

Microarray analyses were conducted on *VvMYB5a* and *VvMYB5b* overexpressing leaves, in order to emphasize differences at transcriptomic level between the transgenic plants and therefore to highlight regulative functions specific for VvMYB5a and VvMYB5b. The sampling was performed following the same criteria of the analysis on *VvMYB5a* / *VvMYB5b* silenced plants. A multiclass comparison analysis was carried out using Significance Analysis of Microarray (SAM) with a false discovery rate (FDR) of 0,2 % for *VvMYB5a* and *VvMYB5b* overexpression. We focused our attention on genes most strongly induced or repressed by choosing a 5-fold cut off for both experiments. Regarding *35S::VvMYB5a* plants, we achieved 552 up-regulated and 860 down-regulated genes, distributed in the 18 functional categories (Supplementary table 3). For *VvMYB5b* overexpression, 585 oligonucleotides presented an increase and 550 presented a decrease in their hybridization signal (Supplementary table 4).

Also for these analyses, the transcripts were annotated using V1 version of the 12X draft annotation of the grapevine genome and grouped in 18 functional categories as reported in figure 10 and 11.

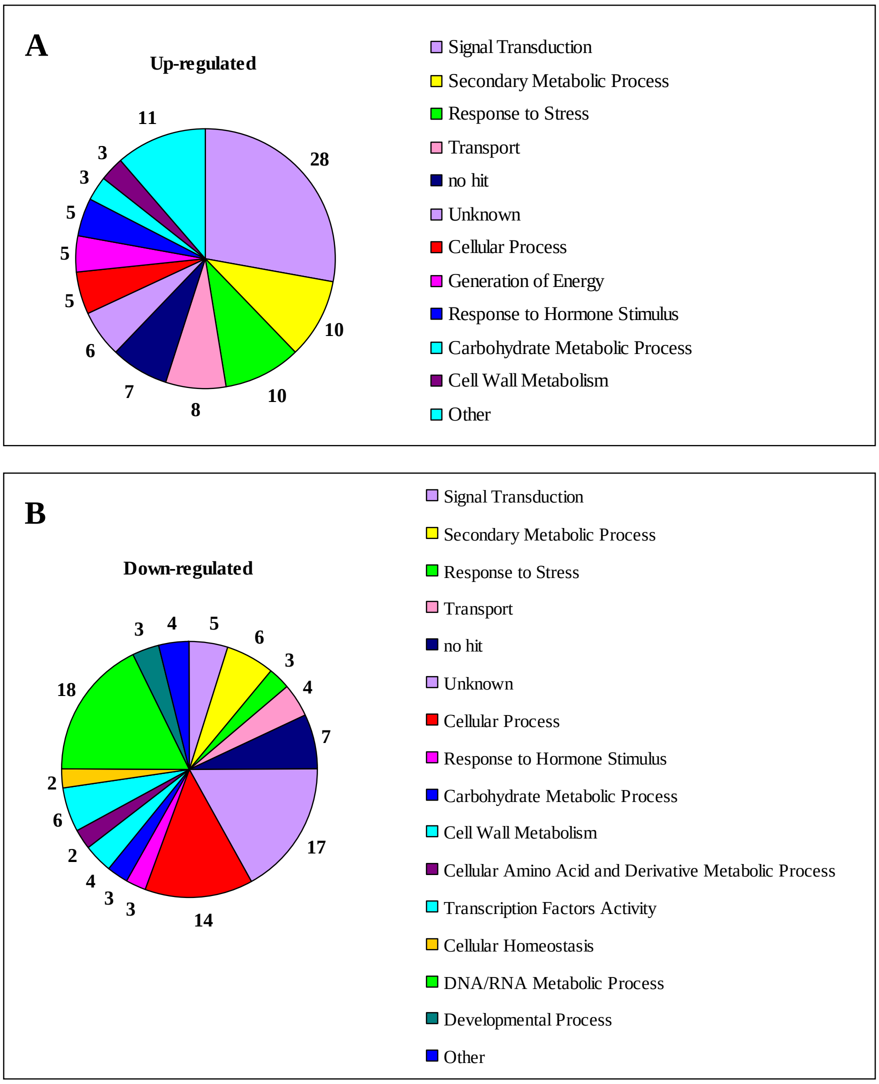

Figure 10. Distribution (%) of up-regulated (**A**) and down-regulated genes (**B**) in *VvMYB5a* overexpressing plant in to 18 Gene Ontology (GO) functional categories.

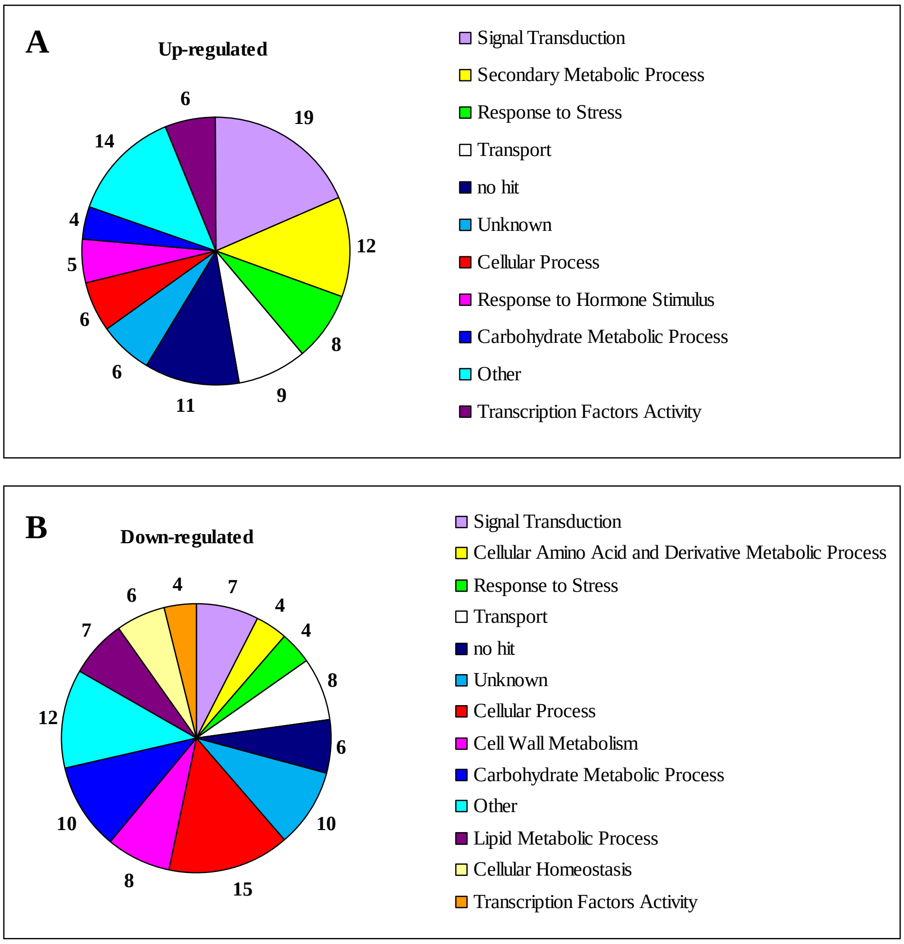

Figure 11. Distribution (%) of up-regulated (**A**) and down-regulated genes (**B**) in *VvMYB5b* overexpressing plant in to 18 Gene Ontology (GO) functional categories.

Table 4 and 5 represent the 50 genes mostly induced in *35S::VvMYB5a and 35S::VvMYB5b* experiment, respectively. The relation between the biological role of these genes and the overexpression of the MYB factors was sometimes difficult to interpret, so we focused on those genes whose function was easy referable to a functional category.

Table 4. The 50 most induced genes in *VvMYB5a* overexpressing leaves compared to the untrasformed line

SEQ_ID	GENE DESCRIPTION	FC
VIT_10s0003g02100	lipase GDSL	68.4
VIT_15s0048g01710	alcohol dehydrogenase	62.3
VIT_18s0041g00390	cysteine protease inhibitor	55.0
VIT_04s0008g04060	RD22	52.8
VIT_05s0029g01140	sucrose-phosphate synthase	45.4
VIT_06s0004g03530	nitrate excretion transporter 1	42.8
VIT_00s0256g00100	no hit	39.9
VIT_17s0000g04400	wall-associated kinase 1 (WAK1)	37.2
VIT_09s0054g01410	beta-amyrin synthase	35.3
VIT_09s0054g01360	cycloartenol synthase	33.5
VIT_06s0004g03520	nitrate excretion transporter 1	30.8
VIT_19s0014g04580	S-locus protein kinase	29.6
VIT_13s0074g00700	ABC transporter G member 22	29.3
VIT_17s0000g04220	chloroplast unusual positioning 1 (CHP1)	29.1
VIT_06s0061g00360	UDP-glucoronosyl and UDP-glucosyl transferase	28.0
VIT_09s0054g01290	beta-Amyrin Synthase	27.6
VIT_09s0002g07830	leucine-rich repeat protein kinase	27.1
VIT_12s0057g00930	Hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyltransferase	26.8
VIT_09s0054g01370	beta-Amyrin Synthase	26.2
VIT_09s0002g03020	light repressible receptor protein kinase	26.0
VIT_12s0057g00870	Cf-2.3	25.8
VIT_11s0016g05430	Unknown protein	24.6
VIT_09s0054g01230	beta-Amyrin Synthase	24.5
VIT_05s0020g02310	pyruvate.orthophosphate dikinase	24.5
VIT_18s0001g14760	lipase 3 (EXL3) family II extracellular	24.4
VIT_09s0002g03010	light repressible receptor protein kinase	24.2
VIT_19s0014g04590	S-locus protein kinase	23.5
VIT_00s0347g00050	Ser/Thr receptor-like kinase1	23.5
VIT_01s0011g04700	heavy-metal-associated domain-containing protein	23.5
VIT_16s0100g00740	no hit	22.8
VIT_16s0022g01330	disease resistance protein (NBS-LRR class)	22.8
VIT_08s0007g00890	Tropinone reductase	22.7
VIT_00s0194g00180	Unknown protein	22.4
VIT_18s0075g00330	sucrose-phosphate synthase	22.4
VIT_05s0062g01160	pectinesterase family	22.0
VIT_18s0001g10610	no hit	21.2
VIT_16s0098g00250	zinc finger (C3HC4-type RING finger)	20.3
VIT_16s0098g00460	lipase class 3	20.3
VIT_18s0001g10630	no hit	19.8
VIT_18s0001g03880	polcalcin	19.8
VIT_13s0074g00660	ABC transporter G member 22	19.7
VIT_17s0000g03380	Calmodulin binding protein	19.3
VIT_13s0074g00690	ABC transporter G member 22	19.1
VIT_09s0002g07750	disease resistance protein	18.9
VIT_02s0025g03310	arsenite transport protein (ArsB)	18.9
VIT_10s0042g00960	DNAJ heat shock N-terminal domain-containing protein	18.8
VIT_07s0104g00070	no hit	18.3
VIT_01s0137g00790	no hit	18.2
VIT_10s0071g00450	S-locus lectin protein kinase	18.2

Table 5. The 50 most induced genes in *VvMYB5b* overexpressing leaves compared to the untrasformed line

SEQ_ID	GENE DESCRIPTION	FC
VIT_00s1240g00010	no hit	74.4
VIT_01s0011g05090	Major latex-like protein 34	67.0
VIT_15s0048g01710	alcohol dehydrogenase	64.4
VIT_02s0012g01380	no hit	50.4
VIT_00s0187g00060	no hit	50.4
VIT_18s0166g00010	receptor kinase	48.7
VIT_18s0041g00390	cysteine protease inhibitor	48.5
VIT_18s0157g00170	alliin lyase precursor	45.5
VIT_15s0046g00490	wax synthase	44.5
VIT_09s0054g01410	beta-amyrin synthase	43.5
VIT_18s0041g00370	double strand break repair protein (XRCC4)	43.3
VIT_09s0002g03070	light repressible receptor protein kinase	43.2
VIT_17s0000g05110	CYP78A4	40.7
VIT_07s0031g01370	flavonoid 3-monooxygenase	39.4
VIT_18s0122g01480	CYP87A2	39.3
VIT_02s0087g00930	9-cis-epoxycarotenoid dioxygenase	38.6
VIT_01s0137g00790	no hit	37.0
VIT_06s0004g01420	glycosyl hydrolase family 1 protein	34.7
VIT_12s0142g00040	Glycerol-3-phosphate acyltransferase 1 (AtGPAT1)	34.5
VIT_02s0025g00700	Aluminum-activated malate transporter 9	32.9
VIT_09s0002g05810	Boron transporter-like protein 4	31.7
VIT_08s0007g07670	NAC domain containing protein 47	30.8
VIT_18s0001g03180	nodulin MtN21 family	30.7
VIT_10s0003g04800	dienelactone hydrolase	30.5
VIT_09s0054g01230	beta-Amyrin Synthase	30.2
VIT_00s0194g00180	Unknown protein	30.1
VIT_19s0015g02910	CYP72A1	29.6
VIT_17s0000g00830	nodulin MtN3 family	29.1
VIT_07s0104g00420	endo-1,3;1,4-beta-D-glucanase precursor	28.3
VIT_07s0031g01380	ferulate 5-hydroxylase	27.8
VIT_18s0001g15660	Pathogen-related	26.9
VIT_16s0098g01170	homeobox-leucine zipper protein HB-12	26.5
VIT_13s0074g00090	glycosyl transferase family 47 protein	26.5
VIT_14s0068g00840	retinoblastoma-related 1 (RBR1)	26.2
VIT_18s0001g00030	CYP87A2	26.2
VIT_08s0040g00920	Glutathione S-transferase 25 GSTU7	25.0
VIT_14s0108g01070	NAC domain containing protein 100	24.1
VIT_06s0004g04210	no hit	23.8
VIT_18s0001g04280	(-)-germacrene D synthase	23.7
VIT_17s0000g09190	octicosapeptide/Phox/Bem1p (PB1) domain-containing protein	23.1
VIT_14s0081g00730	ethylene response factor ERF1	22.4
VIT_06s0061g00180	WD40	22.1
VIT_10s0116g00170	S-locus lectin protein kinase	22.1
VIT_01s0011g04190	no hit	22.0
VIT_04s0023g03230	Auxin-responsive SAUR9	21.5
VIT_04s0044g01150	aminotransferase, class V	21.4
VIT_16s0022g01330	disease resistance protein (NBS-LRR class)	21.4
VIT_18s0001g04480	Germacrene-D synthase	21.2

In both analyses we identified many up-regulated genes related to the "secondary metabolism", such as hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyltransferase, involved in the production of methoxylated monolignols and flavonoid 3'-hydroxylase in the flavonoid synthesis.

Both transcription factors led to the induction of genes related to transport, such as the high affinity K^+ transporter or the cation/H^+ exchanger. The regulation of proton/cation transporters was previously described for the petunia ortholog PH4, involved in the vacuolar acidification and vesicle transport (Quattrocchio *et al.*, 2006; Provenzano, 2011). Our microarray data are in line with the results obtained expressing these MYB factors in petunia and illustrated in chapter 2, suggesting that VvMYB5a and VvMYB5b are regulators of these pathways also in grapevine. Among the transcription factors, it is noteworthy the induction of a homolog to the negative regulator *CAPRICE (CPC)* by VvMYB5b. The modulation of the same *CPC* expression was detected also in microarray analysis on grape hairy roots overexpressing *VvMYB5a*.

Both VvMYB5a and VvMYB5b modulates genes related to the response to stress. It remains to be clarified if this was caused by a direct regulation by VvMYB5a and VvMYB5b or to a particular stress condition of both the transgenic plants. Among these, we noticed a strong induction of many β-amyrin synthase in both analyses. β-amyrin is the precursor of oleanolic acid, the major component of grape berry waxes that function as barrier to the evaporation of water (Grncarevic M., 1971). Some other stress-related genes were specifically detected in each experiment. For example, *35S::VvMYB5a* led to the induction of many stilbene synthases. Some of these genes appeared down-regulated in *VvMYB5a / VvMYB5b* silenced plants. Regarding VvMYB5b, we found genes for germacrene-D synthase and pinoresinol-lariciresinol reductase involved in the defense against pathogens (Lucker *et al.*, 2004; Schroeder *et al.*, 2006).

The overexpression of both *VvMYB5a* and *VvMYB5b* led to the down-regulation of a wide set of genes almost equally distributed in all 18 functional categories. Taken together, our results indicate that the *VvMYB5a* and *VvMYB5b* overexpression interferes with different metabolic pathways. We therefore hypothesized that they are involved in the control of numerous processes, even if further analyses are required as confirmation.

DISCUSSION

The information available on VvMYB5a and VvMYB5b functions has been mainly inferred so far by expression in heterologous systems like tobacco (Deluc *et al.*, 2006; Deluc *et al.*, 2008), tomato (Mahjoub *et al.*, 2009) and petunia (Chapter 2). The use of these species yielded a wide range of effects observed in different organs of the transgenic plants, probably because, when expressed in different genetic backgrounds, VvMYB5a and VvMYB5b take part to processes normally controlled by the orthologous proteins in the species. For this reason, here we conducted functional studies directly in grapevine.

The first approach used was the ectopic expression in hairy roots of *Vitis vinifera* ‘Corvina’. Because of the difficulty encountered with the technique and the recalcitrance of the cultivar ‘Corvina’ to the transformation, only hairy roots overexpressing *VvMYB5a* were obtained. The microarray analysis performed confirmed VvMYB5a as regulator of the early structural genes of the flavonoid pathway, as previously demonstrated by Deluc *et al*. (2008). As expected, the *VvUFGT* gene was not present amongst the modulated genes, being regulated only by VvMYBA1 (Deluc *et al.*, 2008; Cutanda-Perez *et al.*, 2009). Our experiment revealed the induction of *VvFLS1*, *VvLAR1* and *VvANR* genes specific for flavonol and proanthocyanidin synthesis, respectively, suggesting that VvMYB5a is a regulator of the general flavonoid pathway as well as flavonol and PA branches. For some of the flavonoid genes, as chalcone synthase or flavonoid 3',5'-hydroxylase, the induction by VvMYB5a was detected also in previous experiments of transient expression in grape cells and complementation studies in petunia. However, sometimes we noticed differences in results obtained in this microarray analysis and the transient assays performed by Deluc (2008). In the case of *VvANR*, for example, the gene expression is induced by VvMYB5a according to the microarray analysis, while it doesn’t seem to be regulated in the transient assay. It is possible that VvMYB5a regulates *ANR* expression through the presence of another intermediate regulator, given by only direct regulation of expression can be detected by transient expression system.

Our microarray analysis revealed that *VvMYB5a* overexpression affected also genes of the cell wall metabolism, phenylpropanoid pathway, hormone- signalling and photosynthesis. We also identified a class of genes related to the transport that resembles the regulatory mechanism of the homolog PhPH4 in vacuolar acidification and vescicular trafficking in *Petunia hybrida.* Complementation analyses of petunia mutants described in chapter 2 revealed that VvMYB5a and VvMYB5b fulfil PhPH4 function in the regulation of these processes. In *Vitis vinifera,* a similar mechanism of transport across the tonoplast and acidification of the vacuolar lumen could be associated with the transport and storage of many secondary metabolites and solutes produced during the berry development. Recently, the presence of vesicle-like bodies transporting anthocyanins was proved in grapevine, suggesting a similar vesicular trafficking to the vacuole (Gomez *et al.*, 2011).

As second approach, *Vitis vinifera* 'Shiraz' was stably transformed with *Agrobacterium tumefaciens.* The generation of transgenic grapevines to perform functional studies was necessary given the absence of cultivars mutated for *VvMYB5a* and *VvMYB5b*. Because of the high sequence similarity between VvMYB5a and VvMYB5b, the expression of both genes was silenced simultaneously to avoid compensation effects. In fact, the expression in tobacco and petunia gave similar results suggesting a partial functional redundancy in grapevine. This hypothesis is also supported by the similar expression profiles in various vegetative organs. However, in berry this similarity is restricted to the early stages of development, while *VvMYB5a* and *VvMYB5b* expression profiles are divergent during ripening. This may indicate that these genes may play different biological roles in part because in berry tissues they are expressed independently. In order to verify these putative functional differences, we overexpressed *VvMYB5a* and *VvMYB5b* in grapevine with two separate events of transformation.

The *VvMYB5a / VvMYB5b* silencing caused severe modifications of the plant architecture. The transgenic plants presented thick and curly leaves with anthocyanin accumulation in the epidermis. Relaying on VvMYB5a involvement in the regulation of flavonol and PA synthesis as suggested by microarray analysis on hairy roots, we firstly analyzed the expression of these specific genes of the flavonoid pathway. Analyses of real time RT-PCR confirmed the role of VvMYB5a and VvMYB5b in the regulation of this pathway and suggested that the anthocyanin accumulation may be due to a down-regulation of structural genes for flavonol and PA synthesis and to a consequent redirection of the flavonoid intermediates to the anthocyanin production. However, the microarray analysis performed to investigate the whole set of genes modulated by *VvMYB5a / VvMYB5b* silencing revealed neither down-regulation of genes for flavonol and PA branches nor up-regulation of genes for anthocyanin production. The leaf samples were collected when the phenotypic effect of the silencing were not visible yet. It is therefore possible that at that stage of development we could not detect the expression of genes directly responsible for the phenotype observed in the later stages. The anthocyanin accumulation in leaf epidermis could represent a secondary effect of the *VvMYB5a / VvMYB5b* silencing, given the induction of ethylene-related transcription factors, which has been shown to have promotive effect on light-induced anthocyanin synthesis (Craker and Wetherbee, 1973). Alternatively, the pigmentation of leaf epidermis can be interpreted as a plant response to a stress condition. In fact, there is a strong association between anthocyanin biosynthesis and both biotic and abiotic stresses, including fungal and viral pathogens, wounding, temperature extremes, high light, UV radiation, drought and salinity (Chalker-Scott, 1999). *VvMYB5a / VvMYB5b* silencing caused also stunted growth of the plants that showed reduced dimension in comparison to the wild type, maybe due to the down-regulation of auxin-biding proteins determinant in controlling plant growth as emerged from the microarray analysis. It is possible that anthocyanins over-accumulated in leaf epidermis act as negative regulators of auxin transport, modulating the activity of auxin-transporting P-glycoproteins (Brown *et al.*, 2001; Peer and Murphy, 2007). The leaves of the transgenic plants were characterized also by thicker veins, caused by massive lignification as suggested by phloroglucinol-HCl staining.

Taken together, these data may indicate that the abnormal phenotype of *VvMYB5a / VvMYB5b* silencing plants is at least in part due to general stress conditions. With this respect, by analyzing the microarray results it is not possible to distinguish the genes directly controlled by VvMYB5a and VvMYB5b from those modulated by a stress condition of the transgenic plants. Indeed, the silencing caused down-regulation of many genes involved in the response to stress. Among these, we identified genes for the synthesis of stilbenes, that are synthesized after microbial attack as part of both constitutive and inducible defense responses or in response to abiotic stress like UV irradiation (Chong *et al.*, 2009; Wang *et al.*, 2010). Interestingly, the same genes were up-regulated in the *VvMYB5a* overexpressing leaves, indicating VvMYB5a as the specific regulator of these stilbene synthases. VvMYB5b may specifically regulate the synthesis of the lignan pinoresinol, a potential agents for insect control (Schroeder *et al.*, 2006), given by the gene specific for the synthesis resulted down-regulated in *VvMYB5a / VvMYB5b* silencing leaves and up-regulated only in plants overexpressing *VvMYB5b*.

The overexpression of *VvMYB5a* and *VvMYB5b* highlighted different aspects of their regulative functions. *VvMYB5a* overexpression affected only leaf epidermis, characterized by necrotic regions. In addition to this effect, VvMYB5b induced also pleiotropic changes including dwarfism, modified leaf structure and different root pigmentation. Similar results were obtained overexpressing *VvMYB5b* in tomato (Mahjoub *et al.*, 2009). Comparing the microarray data obtained from the overexpressing plants, we distinguished genes regulated by both VvMYB5a and VvMYB5b from those specifically controlled by each transcription factor. *β–amyrin synthase* is a gene common to both analyses, it is the precursor of oleanolic acid, the major component of grape berry waxes that function as barrier to the evaporation of water (Grncarevic M., 1971) and therefore may be related to the resistance to abiotic stresses. VvMYB5a and VvMYB5b are homologs of OsMYB4 that controls the chilling tolerance in rice and enhances tolerance to drought stress, when expressed in tomato (Vannini *et al.*, 2007). Interestingly, the expression of *VvMYB5b* in tomato plants caused modifications in β-amyrin content and consequent changes in cell morphology (Mahjoub *et al.*, 2009). These genes for β–amyrin synthase were not detected in the microarray analysis on *VvMYB5a / VvMYB5b* silenced grapevines, probably because oleanolic acid is absent from the cuticular wax of grapevine leaves and were also absent in microarray analysis performed on *VvMYB5a* hairy roots. Therefore, the functions of VvMYB5a and VvMYB5b in amyrin biosynthesis should be further studied. Other evidence of the involvement in the response to biotic or abiotic stresses is represented by the specific regulation of germacrene synthases by VvMYB5b: in grapevine germacrene is produced in flower and in some vegetative tissues and could function in the formation of volatile or non-volatile terpenoids, providing protection against pathogens (Lucker *et al.*, 2004).

A global analysis of the transcriptomic data revealed a set of genes up- or down-regulated in all MYB transgenic lines, that could be also explained as a plant response to the stable transformation with *Agrobacterium tumefaciens*. The results obtained from microarray analyses on transgenic leaves and hairy roots are sometimes different, probably because of different roles of VvMYB5a and VvMYB5b in these organs. For example, VvMYB5a induced the expression of *WEREWOLF* and *CAPRICE* homologs in hairy roots but not in leaves. The overexpression of a given transcription factor can give different results depending on the tissue considered. It is possible that the effects of *VvMYB5a* / *VvMYB5b* silencing and their independent overexpression on the grape berry will be again different from those observed in vegetative organs.

All our results provided the first real evidence that the role of VvMYB5a and VvMYB5b is not restricted to the flavonoid metabolism but rather they control many aspects of the plant development and metabolism in different organs. The presence of tissue-specific partners could be essential in the formation of multiple complexes and in the definition of different regulatory networks. The regulation of the flavonoid pathway in berry tissues during the development, demonstrated also by previous experiments, cannot be verified at this moment as the transgenic plants are not fruiting yet. The transformation of *Vitis vinifera* gave a wealth of knowledge about VvMYB5a and VvMYB5b roles in grapevine, giving a general overview of their functions and providing the basis for further studies for the definition of the downstream regulated pathways.

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SUPPLEMENTARY DATA

Supplementary table 1. The 80 most induced genes (> 3 fold) in *VvMYB5a* overexpressing hairy roots compared to the untransformed line.

SEQ_ID	GENE DESCRIPTION	FC
VIT_19s0090g01470	protease inhibitor/seed storage/lipid transfer protein (LTP)	51.2
VIT_12s0059g00590	Allergenic protein Pt2L4	37.8
VIT_14s0068g00930	Chalcone synthase	36.7
VIT_08s0007g00330	Metallothionein	33.0
VIT_07s0130g00030	proteinase inhibitor se60	29.3
VIT_14s0068g00040	no hit	29.1
VIT_05s0049g00660	no hit	26.6
VIT_11s0016g01220	Auxin-binding protein ABP19a precursor	23.7
VIT_13s0064g01750	vicianin hydrolase	22.3
VIT_14s0083g00250	no hit	21.0
VIT_03s0063g02340	dehydration-responsive protein (RD22)	20.9
VIT_06s0004g00990	Dirigent protein	20.5
VIT_00s0346g00080	sinapyl alcohol dehydrogenase	20.4
VIT_11s0016g05830	no hit	20.2
VIT_02s0012g01370	no hit	20.0
VIT_13s0064g01660	prunasin hydrolase isoform PHA precursor	19.7
VIT_10s0003g02890	LHCII type I CAB-1	19.2
VIT_13s0064g01640	beta-primeverosidase	18.6
VIT_17s0000g02470	thaumatin	18.1
VIT_10s0003g02900	LHCII type I CAB-1	17.8
VIT_03s0038g02520	aquaporin PIP3	17.7
VIT_19s0014g01060	sesquiterpene synthase	17.1
VIT_06s0004g04120	Aquaporin TIP1;3	17.0
VIT_12s0028g00320	LHB1B1	17.0
VIT_02s0012g01350	no hit	16.7
VIT_10s0116g00500	Myb CAPRICE CPC	16.1
VIT_01s0011g05120	major latex	15.9
VIT_03s0063g00810	CARBOXYESTERASE 12; CXE12	15.4
VIT_15s0048g01200	subtilisin serine endopeptidase (XSP1)	14.2
VIT_18s0001g10040	LRX1 (LEUCINE-RICH REPEAT/EXTENSIN 1)	14.1
VIT_18s0001g07340	aspartic proteinase nepenthesin-1 precursor	13.9
VIT_05s0049g00570	no hit	13.8
VIT_12s0028g02840	isoflavone methyltransferase/Orcinol O-methyltransferase 2 oomt2	13.8
VIT_02s0154g00290	Extensin	13.7
VIT_18s0001g01460	no hit	13.6
VIT_14s0128g00670	germin-like protein 3 [Vitis vinifera]	13.6
VIT_19s0177g00140	thiazole biosynthetic enzyme. chloroplast (ARA6)	13.3
VIT_07s0141g01050	no hit	13.2
VIT_14s0081g00010	IAA16	13.2
VIT_09s0002g01090	flavonoid 3-monooxygenase	12.8
VIT_01s0011g02710	no hit	12.8
VIT_14s0128g00630	germin-like protein 3 [Vitis vinifera]	12.6
VIT_07s0104g01100	peroxidase	12.5
VIT_18s0072g00380	Abscisic stress ripening protein 2 (ASR2)	12.4
VIT_03s0180g00070	cyclase	12.0
VIT_03s0091g01290	serine carboxypeptidase S10	11.9
VIT_16s0022g02270	basic helix-loop-helix (bHLH) family	11.8
VIT_17s0000g03270	CYP77A5P	11.6
VIT_03s0088g00260	serine carboxypeptidase S10	11.3
VIT_11s0016g00960	subtilase	11.3
VIT_05s0049g00610	no hit	11.3
VIT_13s0067g02930	expansin [Vitis labrusca x Vitis vinifera] EXPA8	11.2
VIT_04s0023g00830	calreticulin 3 (CRT3)	11.1
VIT_00s0371g00100	mannitol dehydrogenase	11.1
VIT_18s0001g03910	nitrate reductase 2 (NR2)	10.8
VIT_19s0090g01360	no hit	10.7
VIT_08s0007g02350	harpin-induced protein	10.7
VIT_19s0093g00350	Glutathione S-transferase 25 GSTU25	10.7
VIT_10s0116g01780	cationic peroxidase	10.7
VIT_14s0108g00740	GASA4	10.6
VIT_00s0153g00050	Glutathione S-transferase 8 GSTU8	10.5
VIT_18s0001g05040	pollen Ole e 1 allergen and extensin	10.5
VIT_05s0049g00730	no hit	10.4
VIT_13s0019g00330	Aquaporin TIP1;3	10.2
VIT_00s2032g00010	no hit	10.2
VIT_04s0008g05640	protease inhibitor/seed storage/lipid transfer protein (LTP)	10.1
VIT_06s0004g05770	heat shock protein 17.4 kDa class I	10.0
VIT_05s0136g00260	chalcone synthase	9.9
VIT_00s0527g00030	ARABINOGALACTAN PROTEIN AGP31	9.9
VIT_14s0083g00400	Leaf senescence protein	9.9
VIT_00s0333g00050	DIR1 (DEFECTIVE IN INDUCED RESISTANCE 1)	9.8
VIT_13s0064g00460	Unknown protein	9.8
VIT_08s0007g03240	carbonic anhydrase precursor	9.8
VIT_08s0007g04780	aquaporin GAMMA-TIP	9.7
VIT_03s0017g01140	cytokinin-O-glucosyltransferase 2	9.7
VIT_08s0007g01350	no hit	9.7
VIT_07s0191g00180	homeobox-leucine zipper protein ATHB-6	9.7
VIT_16s0050g01300	C2 domain-containing protein	9.6
VIT_08s0040g01890	aquaporin PIP2;4	9.6
VIT_12s0034g00160	UDP-glucose glucosyltransferase	9.6

Supplementary table 2. Differentially expressed genes (> |3| fold) in *VvMYB5a / VvMYB5b* silenced leaves compared to the untransformed line.

SEQ_ID	GENE DESCRIPTION	FC
VIT_17s0119g00280	alpha-amylase/subtilisin inhibitor	-21.1
VIT_18s0075g00270	no hit	-17.0
VIT_18s0086g00410	auxin-binding protein ABP19	-16.7
VIT_05s0077g01150	Beta-1.3-glucanase	-15.7
VIT_18s0001g00740	ornithine decarboxylase	-15.5
VIT_12s0028g02990	fasciclin arabinogalactan-protein (FLA13)	-14.6
VIT_14s0006g03210	unknown	-13.0
VIT_05s0094g00340	Chitinase class IV	-12.0
VIT_07s0130g00030	proteinase inhibitor se60	-10.6
VIT_16s0100g01030	stilbene synthase [Vitis quinquangularis]	-10.5
VIT_12s0034g01900	globulin-like protein	-10.1
VIT_16s0100g00760	Stilbene synthase 3	-9.7
VIT_12s0034g01920	no hit	-9.6
VIT_17s0000g08460	carbonic anhydrase. chloroplast precursor	-9.3
VIT_19s0027g01880	amino acid transport protein	-8.3
VIT_15s0048g00510	pectinesterase family	-8.1
VIT_08s0105g00380	Leucoanthocyanidin dioxygenase	-7.7
VIT_12s0059g01410	no hit	-7.7
VIT_12s0034g01970	cupin	-6.8
VIT_03s0088g00050	serine carboxypeptitase 1	-6.8
VIT_18s0164g00050	no hit	-6.8
VIT_18s0001g13130	BEL1-LIKE HOMEODOMAIN 10	-6.8
VIT_03s0091g00390	snakin-1	-6.7
VIT_02s0025g00750	pinoresinol forming dirigent protein	-6.6
VIT_07s0005g05930	auxin-binding protein ABP19	-6.5
VIT_18s0075g00460	TIR-NBS-LRR disease resistance	-6.5
VIT_05s0020g02200	inosine-uridine preferring nucleoside hydrolase	-6.3
VIT_16s0100g00960	stilbene synthase [Vitis pseudoreticulata]	-6.3
VIT_16s0100g00940	stilbene synthase 3 [Vitis sp. cv. 'Norton']	-6.1
VIT_07s0031g00770	Unknown protein	-5.8
VIT_03s0088g00110	serine carboxypeptidase SCPL17	-5.8
VIT_00s0228g00060	unknown	-5.8
VIT_18s0001g06140	Phosphate-induced protein 1	-5.6
VIT_00s0480g00040	polyphenol oxidase II. chloroplast precursor	-5.6
VIT_15s0046g00520	wax synthase	-5.5
VIT_02s0025g04330	Thaumatin VVTL1 [Vitis vinifera]	-5.3
VIT_09s0002g01320	germin-like protein	-5.3
VIT_19s0027g01870	Amino acid permease	-5.1
VIT_11s0016g05540	dicyanin	-5.1
VIT_02s0033g01390	no hit	-4.9
VIT_08s0040g01490	unknown	-4.7
VIT_13s0067g00840	TTN10 (TITAN 10)	-4.7
VIT_10s0042g00840	stilbene synthase [Vitis pseudoreticulata]	-4.6
VIT_17s0000g08450	carbonic anhydrase chloroplast	-4.6
VIT_18s0075g00440	TIR-NBS-LRR disease resistance	-4.6
VIT_17s0119g00160	trypsin and protease inhibitor Kunitz family	-4.4
VIT_08s0007g08750	heat shock transcription factor B3	-4.4
VIT_03s0091g00650	no hit	-4.3
VIT_02s0025g00760	pinoresinol forming dirigent protein	-4.3
VIT_17s0000g05580	isopiperitenol dehydrogenase	-4.3
VIT_00s0479g00020	basic helix-loop-helix (bHLH) family	-4.2
VIT_18s0001g06600	Unknown protein	-4.2
VIT_17s0000g09340	no hit	-4.1
VIT_08s0058g01280	no hit	-4.1
VIT_05s0077g01560	pathogenesis protein 10.3 [Vitis quinquangularis]	-4.1
VIT_16s0100g01020	stilbene synthase [Vitis pseudoreticulata]	-4.0
VIT_18s0086g00590	auxin-binding protein ABP19	-4.0
VIT_00s0392g00060	(E.E)-alpha-farnesene synthase	-4.0
VIT_01s0011g03320	ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER AHP2	-4.0
VIT_06s0004g02550	Kiwellin Ripening-related protein grip22	-4.0
VIT_00s0480g00030	polyphenol oxidase	-4.0
VIT_00s0361g00060	(E.E)-alpha-farnesene synthase	-4.0
VIT_03s0038g04720	syntaxin 1B/2/3/4	-3.9
VIT_18s0122g00470	spindle checkpoint protein Bub1b	-3.9
VIT_10s0116g00080	tetratricopeptide repeat (TPR)-containing SET domain protein	-3.9
VIT_19s0014g05330	ankyrin repeat	-3.9
VIT_18s0001g14300	cytomatrix protein	-3.8
VIT_08s0040g01930	Unknown protein	-3.8
VIT_14s0066g01220	ATMYB66/WER/WER1 (WEREWOLF 1)	-3.8
VIT_00s0250g00090	oxidoreductase. 2OG-Fe(II) oxygenase	-3.8
VIT_00s1616g00010	Unknown protein	-3.7
VIT_09s0002g00800	zinc finger (CCCH-type) family protein	-3.7
VIT_06s0004g00210	subtilisin protease	-3.7
VIT_01s0011g03770	no hit	-3.7
VIT_00s0125g00310	Unknown protein	-3.7
VIT_17s0000g01370	cytidine/deoxycytidylate deaminase	-3.7
VIT_10s0003g00430	Unknown protein	-3.7
VIT_07s0129g01080	MICROTUBULE END BINDING PROTEIN 1 (EB1)	-3.7
VIT_00s0199g00230	unknown	-3.7
VIT_12s0028g00860	NAC domain-containing protein 42	-3.7
VIT_15s0046g01110	histone H3.2	-3.6
VIT_01s0010g02460	glyceraldehyde-3-phosphate dehydrogenase. cytosolic 3	-3.6
VIT_11s0037g00910	no hit	-3.6
VIT_18s0001g08870	no hit	-3.6
VIT_04s0023g02900	Ferulate-5-hydroxylase	-3.6
VIT_12s0121g00080	Copine BON3 (BONZAI 3)	-3.5
VIT_05s0077g01550	pathogenesis protein 10.3 [Vitis quinquangularis]	-3.5

VIT_08s0007g08010	no hit	-3.5
VIT_10s0003g00440	isoflavone methyltransferase/ Orcinol O-methyltransferase 1 oomt1	-3.5
VIT_14s0081g00200	AN3 (ANGUSITFOLIA3)	-3.5
VIT_18s0072g00270	unknown	-3.5
VIT_09s0002g01380	ATMYB66/WER/WER1 (WEREWOLF 1)	-3.5
VIT_00s0207g00300	no hit	-3.4
VIT_18s0001g07560	kinesin family member 4/7/21/27	-3.4
VIT_08s0007g06500	Unknown protein	-3.4
VIT_08s0040g00550	Pinoresinol-lariciresinol reductase	-3.4
VIT_14s0006g01340	myb domain protein 113	-3.4
VIT_17s0000g04560	kinetochore protein	-3.4
VIT_08s0007g04950	xyloglucan endotransglucosylase/hydrolase 32	-3.3
VIT_17s0000g04070	unknown	-3.3
VIT_14s0219g00030	zinc finger (C3HC4-type RING finger)	-3.3
VIT_11s0016g02200	Beta-galactosidase	-3.3
VIT_04s0008g02570	REPLICON PROTEIN A (ATRPA2/ROR1/RPA2)	-3.3
VIT_15s0046g00700	wax synthase	-3.3
VIT_05s0077g02200	Unknown protein	-3.3
VIT_11s0016g02780	myb domain protein 68	-3.3
VIT_07s0005g04620	no hit	-3.3
VIT_18s0001g14320	Unknown protein	-3.3
VIT_18s0001g11100	serine carboxypeptidase	-3.3
VIT_06s0009g03600	Wall-associated receptor kinase-like 14	-3.3
VIT_14s0006g02290	DREB sub A-4 of ERF/AP2 transcription factor	-3.2
VIT_07s0129g01010	unknown	-3.2
VIT_04s0023g03220	myosin-related	-3.2
VIT_04s0008g05150	zinc finger (Ran-binding)	-3.2
VIT_06s0004g03290	MaoC-like dehydratase	-3.2
VIT_15s0046g01130	myb TRIPTYCHON	-3.2
VIT_08s0007g03520	purple acid phosphatase 22- ATPAP22/PAP22	-3.2
VIT_13s0147g00100	Nucleic acid-binding. OB-fold	-3.1
VIT_19s0014g01180	pathogenesis-related	-3.1
VIT_17s0000g00430	basic helix-loop-helix (bHLH) family	-3.1
VIT_03s0017g02110	Anthocyanidin 3-O-glucosyltransferase	-3.1
VIT_18s0001g13340	purple acid phosphatase 32- ATPAP32/PAP32	-3.1
VIT_17s0000g00250	Unknown protein	-3.1
VIT_09s0002g04450	helicase; 55525-51977	-3.1
VIT_08s0007g01840	no hit	-3.1
VIT_06s0061g00070	ubiquitin-conjugating enzyme E2 C	-3.1
VIT_16s0039g00730	DNA polymerase delta. subunit D	-3.1
VIT_14s0006g01290	myb domain protein 113	-3.1
VIT_18s0072g00710	Small nuclear ribonucleoprotein LSM Sm-like	-3.1
VIT_08s0007g08840	Glycosyl transferaseHGA1	-3.1
VIT_18s0001g06780	protein kinase CRK1	-3.0
VIT_08s0007g03190	Nucleic acid-binding. OB-fold	-3.0
VIT_10s0116g01340	copper-binding family protein	-3.0
VIT_19s0014g03290	NAC domain containing protein 19	-3.0
VIT_19s0090g01400	Wax synthase isoform 1	3.0
VIT_12s0121g00110	no hit	3.0
VIT_02s0012g03180	unknown	3.0
VIT_12s0034g00330	no hit	3.1
VIT_12s0034g02340	disease resistance protein (NBS class)	3.1
VIT_16s0039g02370	EIX receptor 2	3.1
VIT_12s0059g00080	ankyrin repeat	3.1
VIT_12s0034g02290	CC-NBS-LRR class	3.1
VIT_13s0064g01680	RNA polymerase beta''	3.2
VIT_04s0008g00410	CLAVATA1 receptor kinase (CLV1)	3.2
VIT_04s0069g00630	glutamate receptor 2.8	3.2
VIT_04s0008g00350	CLAVATA1 receptor kinase (CLV1)	3.2
VIT_00s0304g00070	lipid-associated family protein	3.2
VIT_14s0060g01830	Unknown protein	3.2
VIT_14s0108g00550	no hit	3.2
VIT_12s0028g02250	cysteine proteinase	3.2
VIT_13s0047g00810	no hit	3.3
VIT_02s0087g00320	glycosyl transferase family 1 protein	3.3
VIT_04s0069g00330	glutamate receptor 2.8	3.4
VIT_11s0065g00520	small G protein / RhoGAP	3.4
VIT_13s0067g02580	disease resistance protein (NBS-LRR class)	3.4
VIT_14s0060g01840	Unknown protein	3.4
VIT_14s0060g01880	Unknown protein	3.4
VIT_17s0000g01290	RGP1 (REVERSIBLY GLYCOSYLATED POLYPEPTIDE 1)	3.4
VIT_08s0007g01200	S-locus receptor protein kinase	3.4
VIT_07s0031g02160	protein phosphatase 2C DBP	3.5
VIT_14s0060g01870	Unknown protein	3.5
VIT_03s0063g01530	CYP82C4	3.6
VIT_07s0205g00180	basic helix-loop-helix (bHLH) family	3.6
VIT_12s0034g02250	disease resistance protein (NBS class)	3.6
VIT_04s0008g00340	CLAVATA1 receptor kinase (CLV1)	3.6
VIT_13s0064g00110	no hit	3.6
VIT_04s0008g00430	CLAVATA1 receptor kinase (CLV1)	3.7
VIT_09s0002g03220	acid phosphatase	3.7
VIT_09s0002g04610	Unknown protein	3.7
VIT_18s0001g13400	Papain cysteine proteinase isoform I	3.7
VIT_08s0058g01030	li	3.8
VIT_14s0060g01800	no hit	3.8
VIT_09s0002g03520	ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER AHP4	3.9
VIT_02s0087g00330	glycosyl transferase family 1 protein	3.9
VIT_00s1317g00010	gibberellin-regulated protein 4 (GASA4)	3.9
VIT_06s0004g04470	heat shock cognate 70 kDa protein 1	4.0
VIT_16s0115g00220	Myosin-like protein XIA	4.0
VIT_02s0154g00270	no hit	4.1
VIT_13s0101g00310	no hit	4.2
VIT_14s0060g01790	Unknown protein	4.5
VIT_16s0013g01080	Ethylene-responsive transcription factor ERF105	4.5
VIT_12s0059g01590	lipase GDSL	4.7
VIT_08s0007g03060	beta-fructofuranosidase	4.8

VIT_16s0013g01090	Ethylene-responsive transcription factor ERF105	4.8
VIT_06s0004g02690	glycosyl transferase family 2 protein	4.8
VIT_11s0052g01540	Protein TRANSPARENT TESTA 12	4.8
VIT_13s0139g00240	no hit	5.1
VIT_01s0010g01690	no hit	5.7
VIT_02s0087g00280	glycosyl transferase family 1 protein	5.9
VIT_09s0002g06750	ERF (ethylene response factor) sub B-6 of ERF/AP2 transcription factor	6.7
VIT_16s0098g00460	lipase class 3	6.8
VIT_07s0005g01090	ATP binding protein	6.9
VIT_11s0052g01560	MATE efflux family protein	6.9
VIT_07s0031g01680	CYP86A1	8.4
VIT_07s0151g01000	Photosystem I reaction center subunit II (PSAD)	8.4
VIT_16s0148g00090	GASA4	9.1
VIT_03s0180g00290	gibberellin-regulated protein 4 (GASA4)	15.0
VIT_05s0020g00050	no hit	15.5
VIT_06s0004g03520	NITRATE EXCRETION TRANSPORTER1	20.2
VIT_06s0004g03530	NITRATE EXCRETION TRANSPORTER1	28.3

Supplementary table 3. Differentially expressed genes (> |5| fold) in *VvMYB5a* overexpressing plants compared to the untransformed line.

SEQ_ID	GENE DESCRIPTION	FC
VIT_10s0003g02100	lipase GDSL	68.4
VIT_15s0048g01710	alcohol dehydrogenase	62.3
VIT_18s0041g00390	cysteine protease inhibitor	55.0
VIT_04s0008g04060	RD22	52.8
VIT_05s0029g01140	sucrose-phosphate synthase	45.4
VIT_06s0004g03530	NITRATE EXCRETION TRANSPORTER1	42.8
VIT_00s0256g00100	no hit	39.9
VIT_17s0000g04400	wall-associated kinase 1 (WAK1)	37.2
VIT_09s0054g01410	beta-amyrin synthase	35.3
VIT_09s0054g01360	cycloartenol synthase	33.5
VIT_06s0004g03520	NITRATE EXCRETION TRANSPORTER1	30.8
VIT_19s0014g04580	S-locus protein kinase	29.6
VIT_13s0074g00700	ABC transporter G member 22	29.3
VIT_17s0000g04220	CHUP1 (CHLOROPLAST UNUSUAL POSITIONING 1)	29.1
VIT_06s0061g00360	UDP-glucoronosyl and UDP-glucosyl transferase	28.0
VIT_09s0054g01290	beta-Amyrin Synthase	27.6
VIT_09s0002g07830	leucine-rich repeat protein kinase	27.1
VIT_12s0057g00930	Hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyltransferase	26.8
VIT_09s0054g01370	beta-Amyrin Synthase	26.2
VIT_09s0002g03020	light repressible receptor protein kinase	26.0
VIT_12s0057g00870	Cf-2.3	25.8
VIT_11s0016g05430	Unknown protein	24.6
VIT_09s0054g01230	beta-Amyrin Synthase	24.5
VIT_05s0020g02310	pyruvate.orthophosphate dikinase	24.5
VIT_18s0001g14760	lipase 3 (EXL3) family II extracellular	24.4
VIT_09s0002g03010	light repressible receptor protein kinase	24.2
VIT_19s0014g04590	S-locus protein kinase	23.5
VIT_00s0347g00050	Ser/Thr receptor-like kinase1	23.5
VIT_01s0011g04700	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	23.5
VIT_16s0100g00740	no hit	22.8
VIT_16s0022g01330	disease resistance protein (NBS-LRR class)	22.8
VIT_08s0007g00890	Tropinone reductase	22.7
VIT_00s0194g00180	Unknown protein	22.4
VIT_18s0075g00330	sucrose-phosphate synthase	22.4
VIT_05s0062g01160	pectinesterase family	22.0
VIT_18s0001g10610	no hit	21.2
VIT_16s0098g00250	zinc finger (C3HC4-type RING finger)	20.3
VIT_16s0098g00460	lipase class 3	20.3
VIT_18s0001g10630	no hit	19.8
VIT_18s0001g03880	polcalcin	19.8
VIT_13s0074g00660	ABC transporter G member 22	19.7
VIT_17s0000g03380	Calmodulin binding protein	19.3
VIT_13s0074g00690	ABC transporter G member 22	19.1
VIT_09s0002g07750	disease resistance protein	18.9
VIT_02s0025g03310	arsenite transport protein (ArsB)	18.9
VIT_10s0042g00960	DNAJ heat shock N-terminal domain-containing protein	18.8
VIT_07s0104g00070	no hit	18.3
VIT_01s0137g00790	no hit	18.2
VIT_10s0071g00450	S-locus lectin protein kinase	18.2
VIT_18s0001g11430	flavonoid 3-monooxygenase	18.1
VIT_10s0003g04800	dienelactone hydrolase	17.9
VIT_09s0002g03120	leucine-rich repeat protein kinase	17.4
VIT_09s0054g01430	no hit	17.3
VIT_09s0002g07720	leucine-rich repeat family protein	17.3
VIT_10s0071g00440	serine/threonine-protein kinase receptor ARK3	17.0
VIT_19s0014g04530	S-locus receptor kinase	16.9
VIT_13s0074g00680	ABC transporter G member 22	16.9
VIT_04s0008g04180	arsenite transport protein (ArsB)	16.5
VIT_09s0070g00640	disease resistance protein	16.4
VIT_01s0011g05380	pectinesterase family	16.2
VIT_09s0054g01520	beta-amyrin synthase	16.2
VIT_19s0014g04540	S-locus protein kinase	15.7
VIT_19s0014g04080	serine/threonine-protein kinase receptor ARK3	15.6
VIT_18s0001g10480	Unknown protein	15.5
VIT_18s0089g00410	Sucrose-phosphate synthase 1	15.4
VIT_09s0018g00670	HcrVf1 protein	15.1
VIT_18s0001g00030	CYP87A2	14.9
VIT_01s0127g00470	galactinol synthase	14.9
VIT_18s0041g00370	double strand break repair protein (XRCC4)	14.8
VIT_12s0028g01360	PMR5 (POWDERY MILDEW RESISTANT 5)	14.8
VIT_19s0014g04510	S-locus protein kinase	14.8
VIT_18s0075g00340	sucrose-phosphate synthase - like protein	14.7
VIT_06s0061g00970	prolylcarboxypeptidase	14.7
VIT_06s0080g01200	harpin-induced family protein / HIN1 family protein / harpin-responsive family protein	14.6
VIT_17s0000g09190	octicosapeptide/Phox/Bem1p (PB1) domain-containing protein	14.5
VIT_14s0068g01160	cytokinin-repressed protein CR9	14.5
VIT_09s0018g00710	disease resistance family protein	14.5
VIT_08s0007g00750	SRG1 (SENESCENCE-RELATED GENE 1)	14.5
VIT_01s0011g00560	DREPP plasma membrane polypeptide	14.5
VIT_16s0013g01120	Ethylene-responsive transcription factor ERF105	14.3
VIT_04s0023g03230	Auxin-responsive SAUR9	14.1
VIT_04s0044g00650	S-receptor kinase	14.0
VIT_19s0015g02410	Receptor protein kinase	14.0
VIT_05s0049g01100	Glutathione S-transferase 25 GSTU7	13.8
VIT_18s0089g00510	Isopentenyltransferase 1	13.7
VIT_04s0008g06210	Nodulin	13.6
VIT_19s0014g04600	S-locus protein kinase	13.6
VIT_18s0001g06070	Unknown protein	13.6
VIT_18s0089g00630	serine/threonine-protein kinase	13.6

VIT_00s0374g00020	S-locus lectin protein kinase	13.6
VIT_04s0044g00680	S-receptor kinase	13.5
VIT_12s0028g01880	isoflavone methyltransferase/ Orcinol O-methyltransferase 1 oomt1	13.5
VIT_09s0054g01220	cycloartenol synthase	13.4
VIT_06s0004g00590	lysine decarboxylase	13.4
VIT_08s0007g06310	myb family	13.3
VIT_17s0000g08720	RKF2 (RECEPTOR-LIKE SERINE/THREONINE KINASE 2)	13.3
VIT_12s0055g00580	receptor protein kinase	13.2
VIT_12s0028g01940	isoflavone methyltransferase/ Orcinol O-methyltransferase 1 oomt1	13.1
VIT_12s0035g00070	Leucine Rich Repeat receptor-like kinase	13.1
VIT_12s0057g00800	Receptor Like Protein 27	12.9
VIT_12s0028g02760	isoflavone methyltransferase/orcinol O-methyltransferase oomtB	12.9
VIT_02s0025g03120	nodulin MtN21 family	12.9
VIT_16s0148g00180	kinase	12.8
VIT_19s0014g04060	ARK3 (Arabidopsis Receptor Kinase 3)	12.8
VIT_19s0014g04160	ARK3 (Arabidopsis Receptor Kinase 3)	12.6
VIT_19s0015g01720	fructose-bisphosphate aldolase. cytoplasmic isozyme 1	12.4
VIT_01s0011g06490	deoxymugineic acid synthase	12.4
VIT_12s0059g00470	Unknown protein	12.3
VIT_12s0035g01240	no hit	12.2
VIT_08s0040g02180	Mlo3	12.2
VIT_19s0014g04630	S-locus protein kinase	12.1
VIT_07s0104g01260	flavin-containing monooxygenase	12.1
VIT_16s0050g01690	receptor kinase homolog LRK10	12.1
VIT_05s0049g01120	Glutathione S-transferase 25 GSTU7	12.1
VIT_17s0000g05110	CYP78A4	12.0
VIT_12s0028g02710	isoflavone methyltransferase/orcinol O-methyltransferase oomtA	11.9
VIT_12s0028g00920	Glutathione S-transferase 9 GSTF9	11.9
VIT_18s0089g00370	CARBOXYESTERASE18 CXE18	11.9
VIT_12s0028g02810	isoflavone methyltransferase/ Orcinol O-methyltransferase 1 oomt1	11.9
VIT_04s0008g04810	unknown	11.9
VIT_10s0042g00590	Leucine-rich repeat receptor-like protein kinase 1	11.8
VIT_19s0014g04470	S-locus protein kinase	11.8
VIT_05s0049g01090	Glutathione S-transferase 25 GSTU7	11.7
VIT_18s0001g15660	Pathogen-related	11.7
VIT_16s0022g01900	pentatricopeptide (PPR) repeat	11.7
VIT_00s1682g00020	flavonoid 3'-hydroxylase cytochrome P450	11.7
VIT_05s0049g01070	Glutathione S-transferase 19 GSTU1	11.6
VIT_03s0180g00290	gibberellin-regulated protein 4 (GASA4)	11.6
VIT_01s0011g02680	Myb-related protein 3R-1 (Plant c-MYB-like protein 1) MYB3R1	11.5
VIT_09s0054g01440	beta-amyrin synthase	11.5
VIT_03s0132g00390	wall-associated kinase 1 (WAK1)	11.5
VIT_05s0029g01000	HcrVf3 protein	11.5
VIT_17s0000g03370	calmodulin-binding protein	11.4
VIT_12s0142g00760	leucine-rich repeat protein kinase	11.4
VIT_01s0011g06470	galacturonic acid reductase	11.4
VIT_12s0057g00810	serine-threonine protein kinase	11.4
VIT_16s0050g02740	receptor-like protein kinase	11.3
VIT_16s0050g01910	no hit	11.3
VIT_05s0049g01080	Glutathione S-transferase 25 GSTU7	11.3
VIT_16s0098g00010	receptor-like protein kinase	11.2
VIT_08s0058g00060	Receptor Like Protein 26	11.1
VIT_10s0003g00680	glutamate receptor protein	11.1
VIT_06s0004g07210	CCT motif CONSTANS-LIKE	11.1
VIT_19s0015g02420	serine/threonine-protein kinase receptor ARK3	11.0
VIT_18s0089g00700	CYP714A1	11.0
VIT_16s0050g02100	disease resistance	10.9
VIT_02s0025g00190	no hit	10.9
VIT_08s0007g02450	aspartyl protease	10.9
VIT_03s0167g00190	CYP714A1	10.9
VIT_09s0002g06480	CYP81B2v1	10.8
VIT_16s0013g01090	Ethylene-responsive transcription factor ERF105	10.8
VIT_09s0002g02990	light repressible receptor protein kinase	10.8
VIT_18s0001g07950	Oligopeptide transporter 4	10.7
VIT_09s0054g00050	no hit	10.6
VIT_00s0226g00170	no hit	10.6
VIT_05s0062g00250	xyloglucan endotransglucosylase/hydrolase 15	10.6
VIT_19s0014g04410	S-locus protein kinase	10.5
VIT_00s0454g00020	no hit	10.5
VIT_03s0017g01550	CRK10 (CYSTEINE-RICH RLK10)	10.5
VIT_16s0039g01210	Ser/Thr receptor-like kinase1	10.4
VIT_09s0002g07710	Disease resistance protein	10.3
VIT_09s0002g02080	CYP90D2	10.3
VIT_19s0014g04500	receptor kinase ARK3	10.3
VIT_12s0059g02630	ankyrin repeat protein family	10.2
VIT_03s0038g04160	DC1 domain-containing protein	10.2
VIT_14s0108g01000	calcium-binding EF hand	10.1
VIT_16s0013g01780	Derlin-1	10.1
VIT_18s0001g08430	Branched-chain-amino-acid aminotransferase 2. chloroplast precursor (Atbcat-2)	10.0
VIT_09s0054g01470	beta-Amyrin Synthase	10.0
VIT_18s0001g15130	WD40 repeat protein	9.9
VIT_16s0050g01550	HcrVf2 protein	9.9
VIT_11s0103g00520	unknown	9.8
VIT_01s0010g03210	HcrVf1 protein	9.8
VIT_18s0001g15220	Unknown protein	9.8
VIT_04s0044g00310	nodulin MtN21 family	9.8
VIT_00s0294g00070	Ser/Thr receptor-like kinase1	9.6
VIT_19s0015g02910	CYP72A1	9.5
VIT_16s0013g01080	Ethylene-responsive transcription factor ERF105	9.5
VIT_00s0500g00010	no hit	9.4
VIT_12s0035g02090	leucine-rich repeat family protein	9.4
VIT_16s0050g01400	C2 domain-containing protein	9.4
VIT_09s0054g01390	beta-Amyrin Synthase	9.3
VIT_00s0366g00020	CRK10 (CYSTEINE-RICH RLK10)	9.3
VIT_01s0150g00370	C2 domain-containing protein	9.3
VIT_09s0070g00620	leucine-rich repeat family protein	9.2

VIT_02s0012g00170	1.4-alpha-D-glucan maltohydrolase	9.2
VIT_04s0008g05770	CBL-interacting protein kinase 25 (CIPK25)	9.2
VIT_12s0028g02950	isoflavone methyltransferase/ Orcinol O-methyltransferase 1 oomt1	9.1
VIT_16s0013g01000	Ethylene-responsive transcription factor ERF105	9.1
VIT_12s0055g00990	peroxidase	9.0
VIT_02s0025g02850	subtilisin protease	9.0
VIT_17s0000g04880	Dirigent protein	8.9
VIT_19s0014g04780	no hit	8.9
VIT_08s0056g00630	acid phosphatase	8.9
VIT_09s0054g01280	beta-Amyrin Synthase	8.9
VIT_19s0014g04490	S-locus protein kinase	8.9
VIT_19s0090g01620	CYP89H3	8.9
VIT_17s0000g05360	germin	8.9
VIT_19s0014g04040	S-receptor protein kinase	8.8
VIT_18s0001g08450	branched-chain-amino-acid transaminase ATBCAT-2	8.8
VIT_12s0057g00820	Leucine-rich repeat	8.8
VIT_00s0780g00030	no hit	8.8
VIT_08s0040g02190	no hit	8.7
VIT_10s0116g00900	Unknown protein	8.7
VIT_18s0001g08200	MATE efflux family protein ZF14	8.7
VIT_02s0025g00350	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	8.7
VIT_18s0072g00990	leucine-rich repeat protein kinase	8.7
VIT_17s0000g01750	auxin-independent growth promoter	8.7
VIT_03s0063g00350	no hit	8.7
VIT_16s0148g00110	receptor kinase homolog LRK10	8.7
VIT_03s0132g00340	Wall-associated receptor kinase-like 10	8.7
VIT_18s0001g13210	Lectin jacalin	8.7
VIT_19s0014g04090	serine/threonine-protein kinase receptor ARK3	8.6
VIT_08s0058g00450	substrate carrier. Mitochondrial	8.6
VIT_13s0067g03130	WRKY DNA-binding protein 55	8.6
VIT_14s0068g01300	Serine palmitoyltransferase	8.6
VIT_02s0025g01750	cellulose synthase CSLG3	8.6
VIT_14s0066g02670	Leucine Rich Repeat receptor-like kinase	8.6
VIT_12s0059g01640	Unknown protein	8.5
VIT_06s0004g06380	UDP-glucose: anthocyanidin 5.3-O-glucosyltransferase	8.5
VIT_01s0011g05060	Major latex-like protein 34	8.5
VIT_00s2776g00010	S-locus lectin protein kinase	8.5
VIT_00s0253g00160	Methyl jasmonate esterase	8.5
VIT_00s0187g00010	no hit	8.5
VIT_00s0429g00040	aspartic proteinase nepenthesin-1 precursor	8.5
VIT_10s0116g00150	receptor kinase RK20-1	8.5
VIT_00s0762g00030	S-locus lectin protein kinase	8.4
VIT_18s0041g00010	Wall-associated receptor kinase 2	8.4
VIT_04s0008g05750	WRKY DNA-binding protein 18	8.4
VIT_10s0003g00780	Glutamate receptor 3.4	8.4
VIT_11s0016g02860	Nudix hydrolase 8	8.4
VIT_16s0148g00070	receptor-like protein kinase	8.4
VIT_09s0054g00300	disease resistance	8.4
VIT_00s1467g00010	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1	8.3
VIT_18s0001g11470	CyP82A3	8.2
VIT_11s0016g02070	basic helix-loop-helix (bHLH) family	8.2
VIT_17s0000g00270	GT2-like trihelix DNA-binding protein	8.2
VIT_18s0001g00660	high mobility group (HMG1/2) family protein	8.1
VIT_08s0007g00020	Cation/hydrogen exchanger 20 (CHX20)	8.1
VIT_12s0142g00800	leucine-rich repeat protein kinase	8.1
VIT_08s0058g00290	receptor lectin protein kinase	8.1
VIT_11s0118g00580	unknown	8.1
VIT_14s0108g00340	Leucine Rich Repeat receptor-like kinase	8.1
VIT_16s0148g00260	Ser/Thr receptor-like kinase1	8.0
VIT_11s0052g00350	receptor kinase TRKe	8.0
VIT_08s0007g03800	Unknown protein	8.0
VIT_14s0066g02680	leucine-rich repeat protein kinase	8.0
VIT_03s0063g00340	no hit	8.0
VIT_16s0039g01770	major pollen allergen Car b 1 isoforms 1A and 1B	8.0
VIT_16s0013g01760	Derlin-1	8.0
VIT_18s0089g00650	Cf-2.2	7.9
VIT_12s0035g00140	Leucine Rich Repeat receptor-like kinase	7.9
VIT_12s0035g00050	no hit	7.9
VIT_05s0049g01290	protein phosphatase 2C	7.9
VIT_18s0001g11520	flavonoid 3-monooxygenase	7.9
VIT_19s0014g04430	S-locus protein kinase	7.9
VIT_05s0094g00350	Chitinase class IV	7.8
VIT_05s0020g00050	no hit	7.8
VIT_16s0050g01720	receptor serine/threonine kinase PR5K	7.8
VIT_14s0108g00930	unknown protein	7.8
VIT_12s0034g02570	Leucine Rich Repeat receptor-like kinase	7.7
VIT_12s0142g00780	leucine-rich repeat transmembrane protein kinase	7.7
VIT_19s0014g04620	S-locus receptor kinase	7.7
VIT_06s0004g08330	no hit	7.7
VIT_16s0148g00280	receptor kinase homolog LRK10	7.7
VIT_08s0007g02940	NAC domain containing protein 90	7.7
VIT_17s0000g03390	Calmodulin binding protein	7.7
VIT_19s0090g01630	CYP89H3	7.7
VIT_16s0148g00090	GASA4	7.7
VIT_09s0002g03460	taurine dioxygenase	7.6
VIT_18s0001g15230	unknown	7.6
VIT_04s0008g00130	Avr9/Cf-9 rapidly elicited protein 146	7.6
VIT_00s0291g00050	octicosapeptide/Phox/Bem1p (PB1) domain-containing protein	7.6
VIT_09s0002g04500	carnitine/acylcarnitine carrier. Mitochondrial	7.6
VIT_16s0050g02730	receptor-like kinase LRK14	7.6
VIT_18s0001g10330	subtilisin protease	7.6
VIT_15s0024g00770	no hit	7.6
VIT_14s0068g02070	peptidase M48	7.6
VIT_18s0001g09800	S-receptor kinase	7.6
VIT_12s0034g02560	DNA (cytosine-5-)-methyltransferase (ATHIM)	7.5
VIT_06s0004g07270	UDP-glucoronosyl and UDP-glucosyl transferase	7.5

VIT_00s0253g00150	Methyl jasmonate esterase	7.5
VIT_12s0034g02600	Leucine Rich Repeat receptor-like kinase	7.5
VIT_07s0031g01680	CYP86A1	7.5
VIT_02s0025g01780	cellulose synthase CSLG3	7.4
VIT_14s0066g00810	raffinose synthase	7.4
VIT_16s0098g00100	receptor serine/threonine kinase	7.4
VIT_03s0110g00170	Hcr9-OR3A	7.4
VIT_12s0055g00680	leucine-rich repeat protein kinase	7.4
VIT_06s0004g08340	DNA-binding bromodomain-containing protein	7.3
VIT_02s0087g00710	ABA 8'-hydroxylase CYP707A1	7.3
VIT_00s0485g00010	receptor kinase homolog LRK14	7.3
VIT_02s0025g01260	NADPH HC toxin reductase	7.3
VIT_09s0002g03100	light repressible receptor protein kinase	7.3
VIT_12s0028g03510	S-locus lectin protein kinase	7.3
VIT_17s0000g03330	receptor serine/threonine kinase PR5K	7.3
VIT_15s0048g02070	BON2-associated protein (BAP2)	7.2
VIT_18s0001g09770	S-locus receptor protein kinase	7.2
VIT_05s0020g03270	S-receptor kinase	7.2
VIT_00s1830g00010	Ser/Thr receptor-like kinase1	7.2
VIT_18s0001g10910	patatin	7.2
VIT_04s0044g01530	UDP-glucuronic acid:anthocyanin glucuronosyltransferase	7.2
VIT_16s0050g02510	WRKY DNA-binding protein 53	7.2
VIT_19s0014g00250	bile acid:sodium symporter	7.1
VIT_09s0002g00700	dormancy/auxin associated protein	7.1
VIT_14s0006g01600	no hit	7.1
VIT_15s0024g00780	no hit	7.1
VIT_12s0028g02780	flavonoid O-methyltransferase	7.1
VIT_05s0165g00130	Ankyrin repeat family	7.0
VIT_09s0054g00080	Hcr2-p2	7.0
VIT_19s0014g04640	S-locus protein kinase	7.0
VIT_16s0013g01110	Ethylene-responsive transcription factor 5	7.0
VIT_00s0425g00030	receptor serine/threonine kinase	7.0
VIT_17s0000g00830	nodulin MtN3 family	7.0
VIT_14s0066g00610	Unknown protein	7.0
VIT_19s0090g00330	1-acyl-sn-glycerol-3-phosphate acyltransferase	6.9
VIT_06s0004g07280	UDP-glucuronosyl/UDP-glucosyltransferase	6.9
VIT_16s0050g01980	EIX receptor 2	6.9
VIT_00s2847g00010	S-locus lectin protein kinase	6.9
VIT_18s0001g09740	S-locus receptor protein kinase	6.9
VIT_14s0108g00310	Leucine Rich Repeat receptor-like kinase	6.9
VIT_16s0050g01610	UDP-glycosyltransferase 88A4	6.9
VIT_18s0001g09840	S-receptor kinase	6.9
VIT_04s0008g07150	aspartyl protease	6.9
VIT_17s0000g09470	NITRATE TRANSPORTER3.1	6.9
VIT_16s0013g00210	metacaspase 1	6.8
VIT_10s0042g00920	Stilbene synthase	6.8
VIT_07s0104g01730	HAK5 (High affinity K+ transporter 5)	6.8
VIT_04s0044g01430	Unknown protein	6.8
VIT_01s0026g01460	Thioredoxin H-type 2 (Trx-H-2)	6.8
VIT_09s0070g00690	leucine-rich repeat family	6.8
VIT_04s0008g00430	CLAVATA1 receptor kinase (CLV1)	6.8
VIT_07s0005g00060	no hit	6.8
VIT_12s0035g00010	DNA (cytosine-5-)-methyltransferase (ATHIM)	6.8
VIT_14s0108g01250	unknown	6.8
VIT_05s0094g01220	CYP706A6	6.8
VIT_01s0011g03990	Receptor serine/threonine kinase PR5K-l	6.8
VIT_07s0104g00270	Isopentenyltransferase 5	6.8
VIT_03s0038g02550	blue copper-binding protein	6.8
VIT_12s0134g00270	S-locus lectin protein kinase family	6.7
VIT_18s0001g06400	no hit	6.6
VIT_16s0148g00060	receptor kinase homolog LRK10	6.6
VIT_10s0116g01860	MATE efflux family protein	6.6
VIT_07s0151g01000	Photosystem I reaction center subunit II (PSAD)	6.6
VIT_12s0057g00950	no hit	6.6
VIT_19s0014g02180	no hit	6.6
VIT_14s0108g00280	Leucine Rich Repeat receptor-like kinase	6.6
VIT_17s0119g00080	organic cation transport protein OCT1	6.6
VIT_11s0016g05170	nitrate transporter	6.6
VIT_01s0026g02740	no hit	6.6
VIT_00s1206g00010	aspartic proteinase nepenthesin-1 precursor	6.5
VIT_08s0056g00800	myb domain protein 60	6.5
VIT_06s0004g05750	copper transporter 1	6.5
VIT_05s0102g00020	Ankyrin repeat protein	6.5
VIT_18s0001g10620	no hit	6.5
VIT_16s0050g01940	S-locus lectin protein kinase	6.5
VIT_18s0001g11490	CYP82C1p	6.5
VIT_07s0005g00660	Late embryogenis abundant protein 5	6.5
VIT_06s0061g00180	WD40	6.5
VIT_04s0008g00300	CLAVATA1 receptor kinase (CLV1)	6.5
VIT_02s0025g01790	cellulose synthase CSLG3	6.4
VIT_19s0014g04440	S-locus protein kinase	6.4
VIT_16s0100g01040	stilbene synthase - grape	6.4
VIT_08s0007g06560	lectin protein kinase	6.4
VIT_16s0050g01530	HcrVf2 protein	6.4
VIT_14s0066g02660	Hcr2-p1.2	6.4
VIT_17s0000g06920	remorin	6.4
VIT_00s0340g00050	endo-1.4-beta-glucanase KORRIGAN (KOR)	6.4
VIT_01s0011g02540	no hit	6.4
VIT_16s0050g01700	receptor serine/threonine kinase PR5K	6.4
VIT_05s0049g00930	inorganic phosphate transporter 1-4	6.4
VIT_09s0002g06470	CYP81B2v1	6.4
VIT_16s0100g01020	stilbene synthase [Vitis pseudoreticulata]	6.3
VIT_01s0011g03520	CONSTANS-LIKE 16	6.3
VIT_05s0077g00900	no hit	6.3
VIT_17s0000g02330	Receptor protein kinase	6.3
VIT_05s0051g00820	nitrate transporter	6.3

VIT_15s0048g00530	Auxin-responsive SAUR11	6.3
VIT_07s0129g00830	CYP81D2	6.3
VIT_10s0003g04540	CATIONIC AMINO ACID TRANSPORTER 1	6.3
VIT_09s0002g03060	leucine-rich repeat protein kinase	6.3
VIT_07s0095g00660	Curculin (mannose-binding) lectin	6.3
VIT_16s0100g00800	Stilbene synthase 4	6.3
VIT_00s0294g00090	Ser/Thr receptor-like kinase1	6.3
VIT_19s0015g02780	CYP72A59	6.2
VIT_00s0471g00050	ACT DOMAIN REPEAT 1 (ACR1) uridylyltransferase	6.2
VIT_06s0004g05120	ARR1 typeB	6.2
VIT_05s0049g00010	cellulose synthase CSLG2	6.2
VIT_11s0052g01250	xyloglucan endotransglucosylase/hydrolase 23	6.2
VIT_17s0119g00260	nitrate transporter 2	6.2
VIT_00s0743g00020	S-locus lectin protein kinase	6.2
VIT_16s0098g00070	PR5K (PR5-like receptor kinase)	6.2
VIT_16s0100g00960	stilbene synthase [Vitis pseudoreticulata]	6.2
VIT_12s0028g03520	S-receptor kinase	6.2
VIT_00s0388g00020	receptor serine/threonine kinase PR5K	6.2
VIT_03s0017g00670	zinc finger (C3HC4-type RING finger)	6.2
VIT_12s0057g01430	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	6.2
VIT_05s0165g00010	ankyrin repeat	6.2
VIT_09s0070g00710	isopentenyltransferase	6.2
VIT_12s0028g02920	isoflavone methyltransferase/ Orcinol O-methyltransferase 1 oomt1	6.1
VIT_19s0014g04180	Curculin (mannose-binding) lectin	6.1
VIT_16s0022g02440	CARBOXYESTERASE 20 CXE20	6.1
VIT_06s0004g03540	MAPK (MPK3)	6.1
VIT_05s0020g03140	SUGAR TRANSPORTER 13	6.1
VIT_04s0008g00390	CLAVATA1 receptor kinase (CLV1)	6.1
VIT_16s0100g00810	stilbene synthase [Vitis vinifera]	6.1
VIT_16s0148g00380	Receptor kinase LRK10	6.1
VIT_00s2576g00010	phosphoenolpyruvate carboxykinase	6.1
VIT_06s0004g06400	anthocyanidin 3-O-glucosyltransferase	6.1
VIT_17s0000g05320	IFA binding protein	6.1
VIT_16s0100g00920	stilbene synthase - grape	6.1
VIT_12s0035g00020	Leucine Rich Repeat receptor-like kinase	6.0
VIT_00s0253g00170	Methyl jasmonate esterase	6.0
VIT_16s0050g01900	leucine-rich repeat protein kinase	6.0
VIT_18s0072g00980	leucine-rich repeat protein kinase	6.0
VIT_16s0148g00290	Ser/Thr receptor-like kinase1	6.0
VIT_16s0050g02720	receptor kinase homolog LRK10	6.0
VIT_04s0008g00370	CLAVATA1 receptor kinase (CLV1)	6.0
VIT_14s0006g03250	AWPM-19	6.0
VIT_00s0258g00040	Ser/Thr receptor-like kinase1	5.9
VIT_03s0017g01940	no hit	5.9
VIT_16s0050g01890	unknown	5.9
VIT_05s0062g00720	UDP-glucoronosyl/UDP-glucosyl transferase UGT75C1	5.9
VIT_02s0025g03140	nodulin MtN21 family	5.9
VIT_15s0045g01020	disease resistance protein (NBS-LRR class)	5.9
VIT_09s0002g00540	lipase GDSL 1	5.9
VIT_12s0035g00110	Leucine Rich Repeat receptor-like kinase	5.9
VIT_03s0132g00350	Wall-associated receptor kinase-like 10	5.9
VIT_18s0001g08810	Unknown protein	5.9
VIT_09s0054g00910	chaperone BCS1 mitochondrial	5.9
VIT_00s0686g00030	ACT DOMAIN REPEAT 1 (ACR1) uridylyltransferase	5.9
VIT_18s0001g06060	UDP-glycosyltransferase 85A1	5.9
VIT_00s0388g00090	Ser/Thr receptor-like kinase1	5.9
VIT_16s0148g00360	PR5K (PR5-like receptor kinase)	5.9
VIT_10s0042g00890	stilbene synthase 1 [Vitis vinifera]	5.8
VIT_08s0007g08280	remorin	5.8
VIT_04s0043g00370	AMMONIUM TRANSPORTER 1;2	5.8
VIT_12s0028g01390	small heat shock protein ACD31	5.8
VIT_08s0007g01360	Unknown protein	5.8
VIT_17s0000g00940	F-box protein	5.8
VIT_17s0000g08770	Cysteine-rich receptor-like protein kinase 2	5.7
VIT_08s0058g00170	Nudix hydrolase 15. mitochondrial precursor (AtNUDT15)	5.7
VIT_15s0045g00880	disease resistance protein (NBS-LRR class)	5.7
VIT_00s0424g00010	Ser/Thr receptor-like kinase1	5.7
VIT_16s0100g01010	Stilbene synthase	5.7
VIT_13s0067g02500	xanthine/uracil permease	5.7
VIT_14s0006g01610	PMI2 (plastid movement impaired 2)	5.7
VIT_05s0020g02500	Unknown protein	5.7
VIT_12s0028g01650	S-receptor kinase	5.6
VIT_19s0014g02190	tyrosine aminotransferase	5.6
VIT_00s0340g00060	endo-1.4-beta-glucanase KORRIGAN (KOR)	5.6
VIT_18s0001g06890	peroxidase GvPx2b. class III [Vitis vinifera]	5.6
VIT_06s0004g05730	universal stress protein (USP) family protein	5.6
VIT_05s0094g01200	flavonoid 3'-hydroxylase cytochrome P450	5.6
VIT_19s0027g00040	CYP72A59	5.6
VIT_14s0006g02630	Receptor-like kinase ARK1AS	5.6
VIT_14s0128g00780	lipoxygenase	5.6
VIT_12s0034g02590	Leucine Rich Repeat receptor-like kinase	5.6
VIT_15s0046g01460	unknown	5.6
VIT_05s0094g00990	UDP-glucose:salicylic acid glucosyltransferase	5.6
VIT_02s0025g04200	unknown	5.6
VIT_00s2248g00010	rust resistance kinase Lr10	5.5
VIT_11s0016g03080	CLAVATA1 receptor kinase (CLV1)	5.5
VIT_14s0036g00140	CyP82A2	5.5
VIT_16s0100g00860	chalcone synthase	5.5
VIT_17s0000g09570	CYP71A26	5.5
VIT_16s0013g01730	Receptor Like Protein 35	5.5
VIT_01s0026g01870	Avr9/Cf-9 induced kinase 1	5.5
VIT_00s1937g00010	amine oxidase	5.5
VIT_12s0035g02150	FERRIC REDUCTION OXIDASE 7 FRO7	5.5
VIT_03s0038g01410	aquaporin PIP PIP1A	5.5
VIT_16s0100g01170	stilbene synthase 1 [Vitis vinifera]	5.4
VIT_18s0122g01270	Protein kinase APK1B	5.4

VIT_06s0004g05800	phosphatidic acid phosphatase / PAP2	5.4
VIT_06s0080g00640	no hit	5.4
VIT_18s0089g00680	peru 2	5.4
VIT_00s1995g00010	Phosphoenolpyruvate carboxykinase	5.4
VIT_03s0091g00690	SALT TOLERANCE ZINC FINGER	5.4
VIT_02s0025g02020	Abl interactor 3 (ABIL3)	5.4
VIT_03s0038g00660	Unknown protein	5.4
VIT_10s0003g00580	ERF (ethylene response factor) sub B-3 of ERF/AP2 transcription factor (ATERF-2)	5.4
VIT_09s0070g00560	EIX receptor	5.4
VIT_18s0001g14270	gibberellin-regulated protein 1 (GASA1)	5.4
VIT_04s0044g01610	CYP82C1p	5.4
VIT_19s0090g01040	no hit	5.4
VIT_12s0028g02880	isoflavone methyltransferase/ Orcinol O-methyltransferase 1 oomt1	5.3
VIT_16s0148g00040	receptor serine/threonine kinase	5.3
VIT_05s0077g01570	pathogenesis protein 10 [Vitis vinifera]	5.3
VIT_04s0210g00140	AMP-dependent synthetase and ligase	5.3
VIT_03s0063g00450	Alpha-amylase	5.3
VIT_11s0016g00330	pectinesterase family	5.3
VIT_03s0063g00120	wax synthase	5.3
VIT_05s0077g01140	bZIP transcription factor BZIP53	5.3
VIT_06s0004g07310	indole-3-acetate beta-glucosyltransferase	5.3
VIT_13s0019g04220	tryptophan/tyrosine permease family	5.3
VIT_08s0056g01500	unknown protein	5.3
VIT_04s0008g00420	CLAVATA1 receptor kinase (CLV1)	5.3
VIT_01s0011g02520	EXS family protein / ERD1/XPR1/SYG1	5.3
VIT_16s0098g00020	receptor serine/threonine kinase	5.3
VIT_12s0055g00670	no hit	5.3
VIT_07s0129g00240	C2H2 zinc finger protein AZF1	5.3
VIT_01s0026g00880	transducin protein	5.3
VIT_00s0662g00040	Ethylene-responsive transcription factor RELATED TO APETALA2 4	5.3
VIT_01s0011g04830	Extensin	5.3
VIT_07s0031g01110	unknown	5.3
VIT_17s0000g09550	CYP71A26	5.3
VIT_18s0001g06840	Peroxidase GvPx2b class III	5.3
VIT_03s0063g00410	Alpha-amylase	5.3
VIT_12s0028g01030	unknown	5.3
VIT_06s0061g00100	glucan endo-1.3-beta-glucosidase. acidic isoform precursor	5.2
VIT_00s0662g00030	Ethylene-responsive transcription factor RELATED TO APETALA2 4	5.2
VIT_14s0036g00210	CYP89A6	5.2
VIT_12s0057g00780	disease resistance protein	5.2
VIT_04s0008g07250	aspartyl protease	5.2
VIT_12s0028g00340	protein kinase	5.2
VIT_16s0039g01560	PLATZ transcription factor	5.2
VIT_03s0017g00660	nematode resistance-like protein	5.2
VIT_04s0023g03470	DNAJ heat shock N-terminal domain-containing protein	5.2
VIT_02s0025g02180	no hit	5.2
VIT_10s0042g00840	stilbene synthase [Vitis pseudoreticulata]	5.2
VIT_00s0250g00090	oxidoreductase. 2OG-Fe(II) oxygenase	5.2
VIT_10s0042g00930	stilbene synthase [Vitis pseudoreticulata]	5.2
VIT_14s0068g01820	Unknown protein	5.2
VIT_00s0258g00010	receptor serine/threonine kinase	5.2
VIT_00s0424g00030	receptor serine/threonine kinase	5.2
VIT_05s0094g01010	indole-3-acetate beta-glucosyltransferase	5.2
VIT_18s0001g15120	Unknown protein	5.2
VIT_03s0017g01140	cytokinin-O-glucosyltransferase 2	5.2
VIT_04s0008g00890	Bet v I allergen	5.2
VIT_03s0017g01510	N-6 Adenine-specific DNA methylase	5.1
VIT_01s0011g05310	CRR3 (CHLORORESPIRATORY REDUCTION 3)	5.1
VIT_18s0001g11580	CYP82A3	5.1
VIT_06s0080g01040	PR5K (PR5-like receptor kinase)	5.1
VIT_16s0013g00980	Ethylene-responsive transcription factor ERF105	5.1
VIT_04s0008g00460	unknown protein	5.1
VIT_04s0008g00440	CLAVATA1 receptor kinase (CLV1)	5.1
VIT_09s0002g03220	acid phosphatase	5.1
VIT_00s2547g00010	WRKY DNA-binding protein 21	5.1
VIT_05s0077g01310	Aldo-keto reductase	5.1
VIT_14s0006g02260	MATE efflux family protein	5.1
VIT_12s0035g00150	Leucine Rich Repeat receptor-like kinase	5.1
VIT_09s0002g01170	hydrolase. alpha/beta fold family protein	5.0
VIT_01s0011g01980	fasciclin arabinogalactan-protein (FLA21)	5.0
VIT_01s0137g00460	RGLG2 (RING DOMAIN LIGASE2)	5.0
VIT_04s0008g04040	RD22 [Vitis vinifera]	5.0
VIT_11s0206g00110	serine carboxypeptidase S28	5.0
VIT_12s0055g00590	SHR5-receptor-like kinase	5.0
VIT_03s0063g01780	Extra-large G-protein (XLG1)	5.0
VIT_15s0021g00940	senescence-associated protein	5.0
VIT_17s0000g02360	Receptor protein kinase	5.0
VIT_18s0001g09820	S-domain receptor kinase	5.0
VIT_19s0027g01230	Hcr9-9E	5.0
VIT_05s0051g00710	Tubulin beta	-5.0
VIT_01s0011g00150	Unknown protein	-5.0
VIT_09s0002g05150	IAA19	-5.0
VIT_17s0000g04340	Unknown protein	-5.0
VIT_17s0000g03670	Unknown protein	-5.0
VIT_11s0078g00400	Auxin responsive SAUR protein	-5.0
VIT_16s0098g01080	GROWTH-REGULATING FACTOR 7	-5.0
VIT_14s0006g00250	Cysteine-rich repeat secretory protein 60	-5.0
VIT_05s0051g00930	DNA primase large subunit	-5.0
VIT_14s0066g02140	phototropin-2	-5.0
VIT_18s0086g00410	auxin-binding protein ABP19	-5.0
VIT_12s0134g00670	Hydroxycinnamoyl-CoA:anthocyanin 5-glucoside-6-O-hydroxycinnamoyltransferase	-5.0
VIT_13s0067g03550	Centromere protein	-5.0
VIT_16s0098g01350	Oligopeptide transporter 6	-5.0
VIT_04s0008g05830	armadillo/beta-catenin repeat	-5.0
VIT_18s0041g01920	kinesin motor protein	-5.0
VIT_07s0191g00250	exo-1.3-beta-glucanase	-5.0

VIT_17s0000g04950	steroid sulfotransferase	-5.0
VIT_07s0129g00280	Unknown protein	-5.1
VIT_07s0005g04410	subtilase	-5.1
VIT_14s0068g01210	sex determination protein tasselseed-2	-5.1
VIT_08s0007g01490	Unknown protein	-5.1
VIT_08s0007g04180	retrotransposon protein. Ty1-copia subclass	-5.1
VIT_03s0091g01060	cyclin delta-2	-5.1
VIT_00s0218g00050	kinesin motor protein	-5.1
VIT_14s0066g02590	nitrate transporter NRT1-5	-5.1
VIT_18s0001g11050	nodulation protein	-5.1
VIT_08s0007g06910	dirigent pDIR3	-5.1
VIT_18s0001g06650	basic helix-loop-helix (bHLH) family	-5.1
VIT_01s0011g04480	Histone chaperone ASF1B (Anti-silencing 1b)	-5.1
VIT_19s0014g02320	Unknown protein	-5.1
VIT_19s0015g00620	lectin protein kinase	-5.1
VIT_14s0030g00180	Unknown protein	-5.1
VIT_00s0302g00010	Unknown protein	-5.1
VIT_08s0007g06340	Unknown protein	-5.2
VIT_00s0385g00010	linalool synthase	-5.2
VIT_13s0067g00840	TTN10 (TITAN 10)	-5.2
VIT_14s0068g02010	IMP dehydrogenase/GMP reductase	-5.2
VIT_08s0007g04920	zinc finger (C3HC4-type RING finger)	-5.2
VIT_04s0008g05140	TSO1 (CHINESE FOR 'UGLY')	-5.2
VIT_18s0089g00010	disease resistance protein	-5.2
VIT_06s0009g03010	flavonoid 3'.5'-hydroxylase [Vitis vinifera]	-5.2
VIT_02s0025g03230	Fringe protein	-5.2
VIT_03s0088g00060	isoflavone reductase	-5.2
VIT_07s0130g00390	DNA (cytosine-5-)-methyltransferase	-5.2
VIT_00s0271g00010	1.8-cineole synthase. chloroplast. putative OS=Ricinus communis GN=RCOM_0906740 PE=4 SV=1	-5.2
VIT_08s0007g07930	CLAVATA1 receptor kinase (CLV1)	-5.2
VIT_19s0138g00120	1.3-beta-glucan synthase	-5.2
VIT_09s0002g05090	CENP-C	-5.3
VIT_10s0003g01550	IMK2 (INFLORESCENCE MERISTEM RECEPTOR-LIKE KINASE 2)	-5.3
VIT_09s0002g09150	RAE1 RNA export 1 homolog	-5.3
VIT_05s0020g04190	PINHEAD (Protein ZWILLE)	-5.3
VIT_06s0009g03390	no hit	-5.3
VIT_12s0059g00440	no hit	-5.3
VIT_08s0007g05010	Unknown protein	-5.3
VIT_08s0007g02620	Ribosomal protein S17 (RPS17A) 40S	-5.3
VIT_18s0001g14300	cytomatrix protein	-5.3
VIT_02s0154g00080	multi-copper oxidase (SKU5)	-5.3
VIT_15s0048g02610	ribosomal protein P1 acidic 60S	-5.3
VIT_00s0199g00210	cyclin delta-2 (CYCD2)	-5.3
VIT_01s0011g06590	protease inhibitor/seed storage/lipid transfer protein (LTP)	-5.4
VIT_18s0001g09090	NPGR1 (NO POLLEN GERMINATION RELATED 1)	-5.4
VIT_19s0014g04880	(-)-germacrene D synthase	-5.4
VIT_18s0001g02500	Unknown protein	-5.4
VIT_01s0011g04710	copper-binding family protein	-5.4
VIT_08s0007g01850	glycine-rich protein	-5.4
VIT_17s0000g06790	flavin-containing monooxygenase	-5.4
VIT_09s0054g00130	kinesin family member 22	-5.4
VIT_05s0062g01260	no hit	-5.4
VIT_18s0072g00830	forkhead-associated domain-containing protein	-5.4
VIT_14s0083g00940	auxin-independent growth promoter	-5.4
VIT_05s0020g01440	Unknown protein	-5.4
VIT_08s0040g02930	unknown	-5.4
VIT_07s0031g01900	Unknown protein	-5.5
VIT_07s0031g01990	ribonucleotide reductase large subunit A	-5.5
VIT_01s0010g01660	Receptor protein kinase	-5.5
VIT_12s0055g01260	Tubulin folding cofactor TITAN 1	-5.5
VIT_00s0407g00050	no hit	-5.5
VIT_11s0016g05790	no hit	-5.5
VIT_00s0956g00030	DNA-binding protein	-5.5
VIT_09s0070g00210	Metal transporter Nramp1	-5.5
VIT_18s0001g12930	heavy-metal-associated domain-containing protein	-5.5
VIT_03s0038g02980	mechanosensitive ion channel	-5.5
VIT_00s0245g00010	Integral membrane protein	-5.5
VIT_17s0000g02040	Unknown protein	-5.5
VIT_11s0052g01730	1-deoxy-D-xylulose-5-phosphate synthase	-5.5
VIT_00s0324g00100	UDP-glycosyltransferase 85A1	-5.6
VIT_09s0002g08960	EREBP-4	-5.6
VIT_00s0228g00060	unknown	-5.6
VIT_05s0020g03350	SEC14 cytosolic factor	-5.6
VIT_11s0052g01780	1-deoxy-D-xylulose-5-phosphate synthase	-5.6
VIT_07s0005g00120	no hit	-5.6
VIT_02s0025g03480	kinesin motor domain containing protein	-5.6
VIT_10s0042g00830	transcription factor jumonji (jmj) protein	-5.6
VIT_01s0010g00510	Unknown protein	-5.6
VIT_02s0025g02680	GROWTH-REGULATING FACTOR 5	-5.6
VIT_18s0001g03680	protein kinase	-5.6
VIT_13s0019g01290	crossover junction endonuclease MUS81	-5.6
VIT_10s0003g01460	Nucleobase-ascorbate transporter 2 (NAT2)	-5.6
VIT_18s0001g14580	kinesin family member 18/19	-5.6
VIT_08s0007g08010	no hit	-5.6
VIT_14s0060g02330	Actin binding FORMIN HOMOLOGY 1	-5.6
VIT_01s0011g05500	beta-1.3-glucanase	-5.6
VIT_16s0013g02090	F-box family protein	-5.6
VIT_12s0059g01730	Unknown protein	-5.6
VIT_02s0025g01300	Unknown protein	-5.6
VIT_19s0090g00790	F-box family protein	-5.7
VIT_12s0034g00980	Peptidase C50. separase	-5.7
VIT_18s0001g11300	LYS/HIS TRANSPORTER 7 LHT7	-5.7
VIT_19s0085g00900	unknown protein	-5.7
VIT_03s0038g01860	PROLINE-RICH PROTEIN 4	-5.7
VIT_17s0053g00590	kinase interacting protein	-5.7

VIT_19s0014g05330	ankyrin repeat	-5.7
VIT_00s0199g00160	heat shock protein binding	-5.7
VIT_00s1847g00010	Adenine phosphoribosyltransferase	-5.7
VIT_07s0151g00310	NIMA protein kinase	-5.7
VIT_10s0003g03590	beta-amyrin synthase	-5.7
VIT_08s0007g01840	no hit	-5.7
VIT_14s0030g01420	GCN5 N-acetyltransferase (GNAT)	-5.7
VIT_15s0046g01360	no hit	-5.7
VIT_18s0001g01730	Shikimate kinase	-5.7
VIT_08s0058g00270	importin beta-2	-5.8
VIT_06s0004g07040	glutaredoxin	-5.8
VIT_04s0008g00880	HOMEOBOX GENE 1	-5.8
VIT_09s0002g00800	zinc finger (CCCH-type) family protein	-5.8
VIT_01s0011g02420	unknown	-5.8
VIT_08s0007g03780	Unknown protein	-5.8
VIT_07s0005g01300	auxin-induced beta-glucosidase	-5.8
VIT_07s0151g00170	Ankyrin	-5.8
VIT_02s0025g03680	tubulin beta-8 chain	-5.9
VIT_17s0000g01120	Unknown protein	-5.9
VIT_00s1247g00020	Ribosomal protein L25	-5.9
VIT_05s0102g00450	calcium-binding protein	-5.9
VIT_00s0880g00010	basic helix-loop-helix (bHLH) family	-5.9
VIT_05s0077g01780	unknown	-5.9
VIT_14s0219g00230	Pectate lyase	-5.9
VIT_10s0003g04240	tyrosine decarboxylase	-5.9
VIT_10s0003g03670	Unknown protein	-5.9
VIT_00s0434g00010	Unknown protein	-5.9
VIT_14s0108g01300	division regulator MinE. Plastid	-5.9
VIT_00s0188g00120	amino acid permease 2	-6.0
VIT_08s0040g01240	basic helix-loop-helix (bHLH) family	-6.0
VIT_01s0011g03320	ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER AHP2	-6.0
VIT_10s0003g03520	beta-amyrin synthase	-6.0
VIT_05s0029g00640	Retrotransposon	-6.0
VIT_13s0019g03630	no hit	-6.1
VIT_15s0046g01960	hydroquinone glucosyltransferase	-6.1
VIT_06s0009g00810	periplasmic beta-glucosidase precursor	-6.1
VIT_01s0146g00400	Humj1	-6.1
VIT_19s0090g01070	glucan endo-1.3-beta-glucosidase 7 precursor	-6.1
VIT_10s0003g03650	beta-amyrin synthase	-6.1
VIT_04s0008g06590	kinetochore protein NUF2	-6.1
VIT_11s0016g02960	glucan endo-1.3-beta-glucosidase 4 precursor	-6.1
VIT_03s0038g01850	PROLINE-RICH PROTEIN 4	-6.2
VIT_14s0030g01870	NIMA protein kinase	-6.2
VIT_16s0050g02310	no hit	-6.2
VIT_18s0001g14180	armadillo/beta-catenin repeat family protein	-6.2
VIT_09s0002g06110	zinc finger (Ran-binding)	-6.2
VIT_18s0001g08650	growth-regulating factor 1	-6.2
VIT_02s0109g00250	4-coumarate-CoA ligase	-6.2
VIT_04s0023g02230	S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase	-6.2
VIT_00s0256g00110	Ankyrin repeat protein	-6.2
VIT_10s0003g00870	TCP FAMILY TRANSCRIPTION FACTOR 4	-6.2
VIT_15s0046g00640	wax synthase	-6.3
VIT_14s0128g00190	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	-6.3
VIT_18s0001g12280	Unknown protein	-6.3
VIT_19s0090g00770	F-box family protein	-6.3
VIT_03s0063g01950	no hit	-6.3
VIT_14s0083g01100	alpha-1.4-glucan-protein synthase 1	-6.3
VIT_00s0557g00010	integral membrane family protein	-6.3
VIT_07s0151g00240	Ankyrin	-6.3
VIT_11s0052g00160	ATP binding . related	-6.4
VIT_17s0000g00370	serine carboxypeptidase II	-6.4
VIT_11s0118g00600	PLATZ transcription factor	-6.4
VIT_14s0006g03210	unknown	-6.4
VIT_18s0001g05490	no hit	-6.4
VIT_11s0016g01250	Growth-regulating factor 6	-6.4
VIT_06s0004g00720	glucan endo-1.3-beta-glucosidase 4 precursor	-6.5
VIT_12s0028g01710	unknown	-6.5
VIT_10s0042g00100	superoxide dismutase. chloroplast	-6.5
VIT_06s0009g01290	unknown	-6.5
VIT_19s0090g00480	ATP synthase gamma chain 1t (ATPC1)	-6.5
VIT_04s0023g01490	ovate family protein 7 OFP7	-6.5
VIT_00s0779g00020	centromere protein	-6.5
VIT_03s0038g02170	thaumatin	-6.5
VIT_19s0090g00400	zinc finger (C3HC4-type RING finger)	-6.5
VIT_05s0094g00510	Unknown protein	-6.5
VIT_01s0026g02290	Origin recognition complex subunit 6	-6.6
VIT_00s0266g00020	1.8-cineole synthase. chloroplast. putative OS=Ricinus communis GN=RCOM_0906740 PE=4 SV=1	-6.6
VIT_18s0001g11200	unknown	-6.6
VIT_12s0059g01090	early-responsive to dehydration	-6.6
VIT_06s0004g07400	Cation/hydrogen exchanger 24 (CHX24)	-6.6
VIT_04s0044g01680	chromatin remodeling 24	-6.6
VIT_13s0019g05370	Histone H2B	-6.6
VIT_13s0067g00220	Aquaporin TMP-C	-6.6
VIT_03s0088g00260	serine carboxypeptidase S10	-6.6
VIT_18s0001g15460	stearyl acyl carrier protein desaturase	-6.6
VIT_15s0021g02300	squamosa promoter-binding protein 8 (SPL8)	-6.7
VIT_11s0065g00100	carbonic anhydrase	-6.7
VIT_07s0031g03220	patellin-1	-6.7
VIT_07s0031g01930	myb TKI1 (TSL-KINASE INTERACTING PROTEIN 1)	-6.7
VIT_08s0007g04200	LATE MERISTEM IDENTITY1 HB51/LMI1	-6.7
VIT_10s0003g03600	lupeol synthase 2	-6.7
VIT_00s0189g00010	Unknown protein	-6.7
VIT_03s0038g02370	mini-chromosome maintenance protein MCM10	-6.7
VIT_07s0129g00190	binding	-6.7
VIT_00s0391g00030	unknown	-6.8

VIT_06s0061g00680	Unknown protein	-6.8
VIT_13s0019g01680	transducin family protein / WD-40 repeat	-6.8
VIT_00s0264g00010	Unknown protein	-6.8
VIT_12s0028g01830	Peroxidase precursor	-6.8
VIT_02s0025g00730	pinoresinol forming dirigent protein	-6.8
VIT_08s0007g01820	transducin protein	-6.8
VIT_05s0077g02280	Unknown protein	-6.8
VIT_14s0128g00090	kinesin family member 5	-6.8
VIT_00s0663g00010	integral membrane family protein	-6.8
VIT_00s0188g00130	amino acid permease 2	-6.8
VIT_05s0094g00520	Unknown protein	-6.9
VIT_00s0499g00050	chromatin assembly factor-1 (FASCIATA1) (FAS1)	-6.9
VIT_06s0004g01750	polygalacturonase inhibitor protein	-6.9
VIT_13s0158g00320	FAD linked oxidase. N-terminal	-6.9
VIT_08s0007g03760	GROWTH-REGULATING FACTOR 4 AtGRF4	-6.9
VIT_08s0040g03290	Mini-chromosome maintenance protein MCM8	-6.9
VIT_09s0002g07250	no hit	-6.9
VIT_02s0012g01340	ubiquitin-specific protease 20	-6.9
VIT_03s0038g01960	proline-rich family protein	-7.0
VIT_08s0007g05470	no hit	-7.0
VIT_01s0010g03470	Caffeoyl-CoA O-methyltransferase	-7.0
VIT_16s0022g00210	Structural maintenance of chromosomes SMC2	-7.0
VIT_15s0048g01750	fasciclin arabinogalactan-protein (FLA8)	-7.0
VIT_16s0098g01740	Unknown protein	-7.0
VIT_13s0147g00100	Nucleic acid-binding. OB-fold	-7.0
VIT_00s0199g00250	zinc finger homeobox 22	-7.0
VIT_10s0003g02180	exonuclease-1	-7.0
VIT_03s0038g04720	syntaxin 1B/2/3/4	-7.1
VIT_15s0046g02210	zinc finger (C3HC4-type RING finger)	-7.1
VIT_03s0038g00830	ARGONAUTE7 AGO7	-7.1
VIT_12s0028g00530	Cytochrome B561	-7.1
VIT_01s0011g04990	chaperonin	-7.1
VIT_00s0227g00130	A/G-specific adenine glycosylase	-7.1
VIT_13s0019g00780	histone H4	-7.1
VIT_06s0004g08430	ATP-dependent DNA helicase recQ	-7.1
VIT_00s0372g00040	1.8-cineole synthase. chloroplast. putative OS=Ricinus communis GN=RCOM_0906740 PE=4 SV=1	-7.1
VIT_11s0016g00590	invertase/pectin methylesterase inhibitor	-7.1
VIT_00s0572g00020	1.8-cineole synthase. chloroplast. putative OS=Ricinus communis GN=RCOM_0906740 PE=4 SV=1	-7.1
VIT_05s0049g00150	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 3-like 3	-7.1
VIT_00s0188g00140	amino acid permease 4	-7.2
VIT_14s0128g00430	protein kinase NPK1 (ANP1)	-7.2
VIT_00s0802g00020	Unknown protein	-7.2
VIT_07s0005g03390	Unknown protein	-7.2
VIT_07s0005g06110	Pore-forming toxin-like protein Hfr-2	-7.2
VIT_16s0022g01070	chalcone synthase [Vitis vinifera]	-7.2
VIT_06s0004g02570	Kiwellin Ripening-related protein grip22	-7.2
VIT_04s0008g03780	Myb-related protein 3R-1 (Plant c-MYB-like protein 1) MYB3R1	-7.2
VIT_14s0066g01270	alpha-L-fucosidase 2 precursor	-7.2
VIT_11s0016g00950	no hit	-7.2
VIT_01s0150g00460	xyloglucan endotransglucosylase/hydrolase precursor	-7.2
VIT_17s0000g05020	squamosa promoter-binding protein 6 (SPL6)	-7.2
VIT_06s0004g01700	ribosomal protein L6 60S	-7.3
VIT_17s0000g06360	Alpha-expansin 3	-7.3
VIT_04s0069g00990	ulp1 protease . C-terminal catalytic domain containing protein	-7.3
VIT_16s0022g00370	GDP-mannose transporter (GONST1)	-7.3
VIT_18s0072g00290	no hit	-7.3
VIT_08s0032g01060	kinesin family member 22	-7.3
VIT_03s0091g00530	N-acetyltransferase ESCO2	-7.3
VIT_19s0014g01120	curculin (mannose-binding) lectin	-7.3
VIT_04s0008g04530	aminoacyl-tRNA synthetase. related	-7.4
VIT_08s0058g00500	breast cancer susceptibility protein 2b	-7.4
VIT_08s0007g01810	ANTR2 (anion transporter 2	-7.4
VIT_16s0039g01780	DNA replication complex GINS protein PSF2	-7.4
VIT_15s0048g02060	hydrolase. alpha/beta fold	-7.4
VIT_00s0499g00040	FAS1 (FASCIATA 1)	-7.4
VIT_07s0005g06500	Receptor protein kinase	-7.4
VIT_19s0015g00530	fasciclin arabinogalactan-protein (FLA1)	-7.4
VIT_06s0004g02230	Unknown protein	-7.4
VIT_00s0333g00060	Unknown protein	-7.4
VIT_16s0039g02430	no hit	-7.4
VIT_03s0038g02660	Unknown protein	-7.5
VIT_00s1616g00010	Unknown protein	-7.5
VIT_02s0087g00810	SWIB complex BAF60b domain-containing protein	-7.5
VIT_03s0038g01930	peptidyl-prolyl cis-trans isomerase ROC5 (ROTAMASE CYP 5)	-7.5
VIT_01s0244g00100	serine carboxypeptidase S10	-7.5
VIT_12s0059g01410	no hit	-7.5
VIT_04s0079g00220	steroid sulfotransferase	-7.5
VIT_08s0058g01250	Unknown protein	-7.6
VIT_03s0038g03210	LysM domain-containing GPI-anchored protein 2	-7.6
VIT_00s0372g00080	1.8-cineole synthase. chloroplast. putative OS=Ricinus communis GN=RCOM_0906740 PE=4 SV=1	-7.6
VIT_08s0040g02960	no hit	-7.6
VIT_08s0007g00210	Unknown protein	-7.6
VIT_18s0001g02390	diphenol oxidase	-7.6
VIT_14s0006g01670	CYP96A10	-7.6
VIT_18s0001g05380	TTL1 (TETRATRICOPETIDE-REPEAT THIOREDOXIN-LIKE 1)	-7.6
VIT_11s0016g02770	meiotic nuclear divisions 1	-7.7
VIT_05s0029g00320	no hit	-7.7
VIT_05s0077g01360	myb domain protein 111	-7.7
VIT_07s0151g00030	Ankyrin	-7.7
VIT_08s0007g06170	Anthranilate phosphoribosyltransferase	-7.7
VIT_01s0010g00240	CTP-synthetase	-7.7
VIT_11s0016g03750	Myb-related protein 3R-1 (Plant c-MYB-like protein 1) MYB3R1	-7.7

VIT_00s0323g00050	invertase/pectin methylesterase inhibitor	-7.7
VIT_13s0084g00820	pepsin A	-7.8
VIT_05s0077g02300	UDP-glucuronic acid decarboxylase 2	-7.8
VIT_00s0385g00020	linalool synthase	-7.8
VIT_01s0011g03860	RKL1 (Receptor-like kinase 1)	-7.8
VIT_12s0055g01140	lipid transfer protein	-7.9
VIT_18s0001g14560	unknown	-7.9
VIT_14s0068g01520	double-stranded RNA-binding (DsRBD) domain-containing protein	-7.9
VIT_07s0129g00150	BTB/POZ domain-containing protein	-7.9
VIT_00s0199g00080	Unknown protein	-7.9
VIT_08s0040g02680	Unknown protein	-7.9
VIT_03s0038g02050	Dirigent protein pDIR7	-7.9
VIT_00s0125g00200	no hit	-7.9
VIT_07s0151g00020	Ankyrin	-7.9
VIT_07s0005g05520	pectate lyase	-7.9
VIT_15s0046g00590	wax synthase	-7.9
VIT_09s0054g00640	N-acetyltransferase ESCO1	-8.0
VIT_12s0059g02420	Peroxidase ATP11A (gb|X98802).	-8.0
VIT_06s0004g03110	unknown	-8.0
VIT_00s1312g00010	basic helix-loop-helix (bHLH) family	-8.0
VIT_08s0040g01970	fasciclin arabinogalactan-protein (FLA11)	-8.0
VIT_18s0075g00270	no hit	-8.1
VIT_18s0001g12030	uracil-DNA glycosylase	-8.1
VIT_11s0065g00720	serine carboxypeptidase S10	-8.1
VIT_00s0522g00020	1.8-cineole synthase. chloroplast. putative OS=Ricinus communis GN=RCOM_0906740 PE=4 SV=1	-8.1
VIT_02s0025g04960	Unknown protein	-8.1
VIT_12s0142g00620	RIC7 (ROP-INTERACTIVE CRIB MOTIF-CONTAINING PROTEIN 7)	-8.2
VIT_18s0001g13540	glycine-rich protein	-8.2
VIT_07s0151g00440	AP2 domain containing protein	-8.2
VIT_17s0000g02460	WUSCHEL RELATED HOMEOBOX 1	-8.2
VIT_00s0372g00020	linalool synthase	-8.2
VIT_12s0055g00930	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	-8.2
VIT_16s0039g02400	protein kinase	-8.2
VIT_18s0001g03080	peptidoglycan-binding LysM domain-containing protein	-8.2
VIT_10s0003g02170	subtilase	-8.3
VIT_13s0064g00980	telomerase reverse transcriptase (TERT)	-8.3
VIT_04s0008g04710	beta-ketoacyl-CoA synthase	-8.3
VIT_13s0019g03610	RNA recognition motif (RRM)-containing protein	-8.3
VIT_10s0003g02120	lipase GDSL	-8.3
VIT_06s0004g04370	histone H4	-8.3
VIT_06s0004g01320	Unknown protein	-8.3
VIT_16s0050g02260	Unknown protein	-8.3
VIT_00s1428g00010	ankyrin repeat	-8.4
VIT_18s0001g07800	kinesin motor protein	-8.4
VIT_05s0094g01430	Unknown protein	-8.4
VIT_13s0064g01440	SYN3 (Sister chromatid cohesion 1 protein 3)	-8.4
VIT_05s0029g00190	glucan endo-1.3-beta-glucosidase 4 precursor	-8.4
VIT_01s0137g00720	lipase GDSL	-8.4
VIT_06s0009g00480	Aluminium-tolerance ALMT1	-8.4
VIT_18s0001g14990	dual-specific kinase DSK1	-8.4
VIT_07s0095g00770	Condensin complex subunit 3 . related	-8.4
VIT_00s0704g00040	calmodulin-binding	-8.4
VIT_15s0046g00660	wax synthase	-8.5
VIT_02s0025g00550	Unknown protein	-8.5
VIT_00s0199g00230	unknown	-8.5
VIT_10s0003g02220	subtilisin	-8.5
VIT_16s0013g00300	ATP-dependent DNA helicase	-8.6
VIT_18s0001g00740	ornithine decarboxylase	-8.6
VIT_17s0000g02470	thaumatin	-8.6
VIT_10s0003g05230	Tetratricopeptide repeat domain male sterility MS5	-8.6
VIT_15s0046g01110	histone H3.2	-8.6
VIT_02s0234g00070	Unknown protein	-8.6
VIT_11s0037g00910	no hit	-8.7
VIT_18s0122g01130	BRCA1 interacting protein C-terminal helicase 1	-8.7
VIT_09s0002g00550	lipase GDSL 1	-8.7
VIT_02s0025g02720	Unknown protein	-8.7
VIT_17s0000g09820	DNA polymerase III gamma and tau subunit	-8.7
VIT_14s0066g01590	NHL repeat-containing protein	-8.7
VIT_02s0025g01290	Esterase	-8.8
VIT_17s0000g01260	squamosa promoter-binding protein 13 (SPL13)	-8.8
VIT_13s0019g01840	cell division cycle 20-like protein 1	-8.8
VIT_14s0083g00050	DDT domain-containing protein	-8.8
VIT_01s0011g03770	no hit	-8.9
VIT_03s0063g02510	protein kinase	-8.9
VIT_06s0061g01610	DNA polymerase epsilon. subunit A	-8.9
VIT_07s0104g00050	Unknown protein	-8.9
VIT_07s0129g00070	BTB/POZ domain-containing protein	-8.9
VIT_01s0010g03550	Nuclear transcription factor Y subunit B related	-8.9
VIT_08s0040g00480	no hit	-8.9
VIT_15s0021g02290	squamosa promoter-binding protein 2 (SPL2)	-9.0
VIT_07s0031g00770	Unknown protein	-9.0
VIT_03s0038g00150	beta-D-galactosidase	-9.0
VIT_07s0031g02770	Unknown protein	-9.1
VIT_07s0005g02550	IAA30	-9.1
VIT_00s0847g00020	myrcene synthase	-9.1
VIT_13s0067g01530	BUB family protein kinase	-9.1
VIT_01s0026g02620	expansin [Vitis labrusca x Vitis vinifera] EXPA15	-9.1
VIT_15s0046g03190	myb domain protein 17	-9.1
VIT_00s0662g00020	Unknown protein	-9.2
VIT_17s0000g01960	Origin recognition complex subunit 5. related	-9.2
VIT_08s0007g07990	no hit	-9.2
VIT_16s0022g01190	chalcone synthase [Vitis vinifera]	-9.2
VIT_18s0001g05060	2.3-bisphosphoglycerate-dependent phosphoglycerate mutase	-9.2
VIT_08s0007g03430	germin	-9.3
VIT_00s0199g00120	heat shock protein binding	-9.3

VIT_18s0001g02290	diphenol oxidase	-9.3
VIT_10s0003g03250	Unknown protein	-9.3
VIT_18s0001g05160	glycosyl hydrolase family 3 protein	-9.3
VIT_00s1235g00010	Unknown protein	-9.3
VIT_16s0039g01450	GROWTH-REGULATING FACTOR 5	-9.3
VIT_14s0068g00190	no hit	-9.4
VIT_13s0147g00230	Unknown protein	-9.4
VIT_03s0038g03550	Unknown protein	-9.4
VIT_11s0052g00840	AP2-like AIL5 (AINTEGUMENTA-LIKE 5)	-9.4
VIT_10s0116g01680	ankyrin repeat	-9.4
VIT_13s0106g00330	lipase GDSL	-9.4
VIT_09s0002g03470	Unknown protein	-9.5
VIT_04s0023g03760	Unknown protein	-9.5
VIT_01s0150g00140	ORTH2/VIM1 (VARIANT IN METHYLATION 1)	-9.5
VIT_09s0002g09020	no hit	-9.5
VIT_14s0108g00440	Tubulin alpha chain	-9.6
VIT_11s0016g05280	peroxidase	-9.6
VIT_05s0049g01350	Kinesin phragmoplast-associated kinesin-related protein 2 (PAKRP2)	-9.6
VIT_16s0039g00430	cyclin-dependent protein kinase CDT1A	-9.6
VIT_00s0215g00010	lateral organ boundaries DOMAIN 6	-9.6
VIT_05s0020g03880	TSO1 (CHINESE FOR 'UGLY')	-9.6
VIT_00s0516g00020	calcium-binding EF hand family	-9.7
VIT_15s0021g00210	EMB2411	-9.7
VIT_14s0006g01340	myb domain protein 113	-9.7
VIT_16s0022g01020	chalcone synthase [Vitis vinifera]	-9.8
VIT_12s0142g00350	membrane protein ycf1	-9.8
VIT_07s0151g00220	Ankyrin	-9.8
VIT_11s0052g00770	serine carboxypeptidase SCPL13	-9.8
VIT_14s0006g00520	glucan endo-1.3-beta-glucosidase 3 precursor	-9.9
VIT_11s0016g05800	no hit	-9.9
VIT_00s0333g00040	early tobacco anther 1. putative	-9.9
VIT_06s0009g02710	DUTP pyrophosphatase	-9.9
VIT_08s0007g00220	Unknown protein	-9.9
VIT_13s0074g00500	Cellulose synthase CSLA09	-9.9
VIT_07s0104g01340	nodulin MtN3 family	-10.0
VIT_19s0014g05070	kinesin family member C1	-10.0
VIT_17s0000g02690	exonuclease. putative	-10.0
VIT_11s0103g00160	no hit	-10.0
VIT_00s0245g00040	no hit	-10.0
VIT_18s0001g15420	auxin efflux carrier protein 6	-10.0
VIT_07s0005g00630	NAD+ ADP-ribosyltransferase	-10.0
VIT_04s0023g01840	GATA transcription factor 19	-10.0
VIT_05s0020g03130	plastocyanin domain-containing protein	-10.1
VIT_07s0031g00020	phosphoric monoester hydrolase	-10.1
VIT_03s0091g00650	no hit	-10.1
VIT_09s0002g01680	calmodulin-binding region IQD6	-10.1
VIT_01s0010g02560	remorin	-10.1
VIT_18s0001g06600	Unknown protein	-10.1
VIT_18s0122g00470	spindle checkpoint protein Bub1b	-10.1
VIT_06s0004g06300	cell division cycle 6	-10.2
VIT_09s0070g00480	LRX2 (LEUCINE-RICH REPEAT/EXTENSIN 2)	-10.2
VIT_00s0245g00030	integral membrane family protein	-10.2
VIT_14s0219g00030	zinc finger (C3HC4-type RING finger)	-10.2
VIT_03s0063g02240	PLATZ transcription factor	-10.2
VIT_04s0043g00940	MONOPTEROS	-10.2
VIT_06s0004g06730	microsomal omega-3 fatty acid desaturase	-10.2
VIT_13s0019g02020	Thaumatin	-10.3
VIT_00s0275g00030	ycf1	-10.3
VIT_13s0019g03430	anthocyanin 5-aromatic acyltransferase	-10.4
VIT_00s0572g00010	linalool synthase	-10.4
VIT_14s0081g00520	ERF12	-10.4
VIT_13s0084g00080	MAP65/ASE1; t-snare	-10.4
VIT_08s0058g01420	Unknown protein	-10.4
VIT_14s0108g00670	chromosome condensation protein	-10.4
VIT_05s0051g00490	outer membrane protein	-10.4
VIT_15s0048g00580	PLATZ transcription factor	-10.5
VIT_17s0000g03290	kinesin motor HIK (HINKEL)	-10.5
VIT_09s0002g06590	UDP-glucose:flavonoid 7-O-glucosyltransferase	-10.5
VIT_00s0218g00140	anthocyanidine rhamnosyl-transferase	-10.5
VIT_08s0007g00440	alpha-expansin 6 precursor	-10.6
VIT_05s0094g00240	Chitinase. class IV [Vitis vinifera]	-10.7
VIT_19s0014g04900	(-)-germacrene D synthase	-10.7
VIT_03s0063g00210	Receptor protein kinase	-10.8
VIT_00s0194g00280	Cyclin D3_1	-10.8
VIT_08s0040g02430	Unknown protein	-10.8
VIT_00s0499g00020	Unknown protein	-10.9
VIT_05s0077g01370	Unknown protein	-10.9
VIT_19s0090g01050	endo-beta-1.4-glucanase	-10.9
VIT_10s0116g01380	amino acid permease 2	-11.0
VIT_07s0005g05180	no hit	-11.0
VIT_09s0002g01350	GROWTH-REGULATING FACTOR 5	-11.0
VIT_04s0069g00520	no hit	-11.0
VIT_18s0001g09580	Cyclin A3;1	-11.1
VIT_13s0067g02030	IMK2 (INFLORESCENCE MERISTEM RECEPTOR-LIKE KINASE 2)	-11.1
VIT_18s0122g00250	diphenol oxidase	-11.1
VIT_15s0048g00650	Unknown protein	-11.2
VIT_01s0011g04400	origin recognition complex subunit 4	-11.2
VIT_18s0001g13930	Auxin response factor 5 (Transcription factor MONOPTEROS)	-11.2
VIT_08s0007g05160	Flavonoid 3'.5'-hydroxylase	-11.2
VIT_02s0025g02690	calmodulin-binding protein	-11.2
VIT_12s0057g00240	Unknown protein	-11.3
VIT_00s0372g00070	linalool synthase	-11.3
VIT_08s0007g05340	kinesin motor protein	-11.3
VIT_11s0016g03970	ubiquitin-conjugating enzyme E2 D/E	-11.3
VIT_18s0001g10740	origin recognition complex subunit 2	-11.4
VIT_14s0066g00650	transducin family protein / WD-40 repeat	-11.4

VIT_04s0044g00860	Protein BRUSHY 1 (Protein TONSOKU) (Protein MGOUN 3)	-11.5
VIT_07s0129g00660	indole-3-acetic acid-amido synthetase GH3.2	-11.5
VIT_03s0017g01970	Unknown protein	-11.5
VIT_18s0001g10710	transcriptional factor B3	-11.6
VIT_17s0053g01040	no hit	-11.6
VIT_04s0008g05150	zinc finger (Ran-binding)	-11.6
VIT_07s0005g01280	BRCT domain-containing protein	-11.6
VIT_07s0005g00740	Endo-1.4-beta-glucanase	-11.6
VIT_17s0000g07440	replication protein A 70 kDa DNA-binding subunit	-11.7
VIT_16s0050g00670	kinesin motor	-11.7
VIT_10s0116g01600	monocopper oxidase SKS4 (SKU5 Similar 4)	-11.8
VIT_07s0005g02580	zinc finger (DHHC type)	-11.8
VIT_12s0134g00090	R-linalool synthase	-11.9
VIT_17s0000g04560	kinetochore protein	-11.9
VIT_19s0027g00220	Unknown protein	-11.9
VIT_07s0104g00470	Unknown protein	-12.0
VIT_19s0015g01500	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	-12.1
VIT_04s0023g02980	pectinesterase family	-12.1
VIT_08s0007g08000	no hit	-12.1
VIT_09s0002g01370	AP2 AINTEGUMENTA	-12.3
VIT_00s0494g00010	GROWTH-REGULATING FACTOR 1	-12.3
VIT_03s0091g00550	Unknown protein	-12.3
VIT_19s0015g01710	kinesin family member 22	-12.3
VIT_11s0016g01550	ankyrin repeat protein family	-12.3
VIT_09s0054g01700	C2-HC type zinc finger protein C.e-MyT1	-12.3
VIT_10s0042g00470	carbonic anhydrase	-12.4
VIT_13s0019g04420	BTB/POZ; NPH3	-12.4
VIT_02s0025g02700	glutaredoxin family protein	-12.4
VIT_11s0016g04090	DNA repair protein MutS	-12.5
VIT_03s0038g02180	glycosyl hydrolase family 10 protein	-12.5
VIT_19s0090g01340	no hit	-12.6
VIT_08s0007g08020	no hit	-12.6
VIT_00s0203g00160	Cyclin D-type	-12.7
VIT_18s0001g10380	heat shock transcription factor B4	-12.7
VIT_11s0016g01320	myb domain protein 13	-12.7
VIT_11s0016g02970	MAPKK NQK1	-12.7
VIT_12s0134g00140	R-linalool synthase	-12.8
VIT_01s0010g02690	DNA polymerase alpha. subunit B	-12.8
VIT_00s0266g00010	1.8-cineole synthase. chloroplast. putative OS=Ricinus communis GN=RCOM_0906740 PE=4 SV=1	-12.9
VIT_07s0005g01550	polygalacturonase PG1	-13.0
VIT_07s0005g01030	Cellulose synthase CSLD5	-13.0
VIT_18s0001g12360	Deoxyuridine 5'-triphosphate nucleotidohydrolase	-13.1
VIT_07s0151g00680	tetratricopeptide repeat (TPR)-containing	-13.1
VIT_15s0021g00880	ferredoxin-related	-13.1
VIT_04s0008g02220	sterile alpha motif (SAM) domain-containing protein	-13.2
VIT_13s0067g02750	no hit	-13.2
VIT_05s0020g01160	binding	-13.2
VIT_00s0271g00060	linalool synthase	-13.3
VIT_16s0022g02030	ERL1 (ERECTA 1)	-13.4
VIT_07s0005g01160	Eukaryotic translation initiation factor 4B	-13.4
VIT_14s0006g01680	CYP96A10	-13.4
VIT_13s0067g02400	myosin heavy chain	-13.4
VIT_14s0036g00830	no hit	-13.5
VIT_16s0039g00480	no hit	-13.6
VIT_14s0219g00090	Unknown protein	-13.6
VIT_10s0003g05680	CHUP1 (CHLOROPLAST UNUSUAL POSITIONING 1)	-13.6
VIT_02s0025g01350	primase polypeptide 1	-13.7
VIT_05s0124g00100	no hit	-13.7
VIT_11s0016g00340	DNA repair protein RAD51	-13.7
VIT_12s0057g00500	Thymidine kinase	-13.7
VIT_01s0010g02590	ATP-dependent DNA helicase RecQ	-13.8
VIT_06s0004g05930	Proliferating cell nuclear antigen (PCNA)	-13.8
VIT_18s0164g00050	no hit	-13.8
VIT_19s0014g03220	Unknown protein	-13.9
VIT_13s0106g00560	no hit	-13.9
VIT_11s0149g00190	transducin family protein / WD-40 repeat	-13.9
VIT_19s0027g01880	amino acid transport protein	-14.0
VIT_19s0015g01530	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	-14.0
VIT_18s0122g00980	glucan endo-1.3-beta-glucosidase 7 precursor	-14.0
VIT_19s0014g00260	Protein kinase PKN/PRK1	-14.0
VIT_06s0004g05920	Proliferating cell nuclear antigen (PCNA)	-14.0
VIT_14s0006g00050	Transposase. IS4	-14.1
VIT_10s0003g03080	minichromosome maintenance protein 3	-14.1
VIT_05s0020g01990	EMB3013	-14.2
VIT_11s0052g00570	nodulin MtN21	-14.4
VIT_14s0128g00450	kinesin motor protein	-14.4
VIT_13s0073g00520	glutaredoxin family protein	-14.4
VIT_01s0137g00050	myb family	-14.4
VIT_12s0034g01140	plastocyanin domain-containing protein	-14.4
VIT_13s0067g03020	Unknown protein	-14.4
VIT_00s0532g00020	kinesin family member C1	-14.4
VIT_14s0108g01630	Unknown protein	-14.5
VIT_08s0058g00330	unknown	-14.5
VIT_00s0522g00010	linalool synthase	-14.5
VIT_11s0016g00460	Kinesin TETRASPORE (NACK1)	-14.5
VIT_08s0007g08030	no hit	-14.6
VIT_10s0092g00360	VPS2.2 SNF7	-14.6
VIT_01s0010g03510	Caffeoyl-CoA O-methyltransferase	-14.6
VIT_00s0271g00030	myrcene synthase	-14.6
VIT_01s0011g02550	high mobility group HMG1/2	-14.7
VIT_01s0010g02240	Unknown protein	-14.7
VIT_05s0029g00400	Kinesin PAKRP1L	-14.7
VIT_12s0057g01510	Unknown protein	-14.8
VIT_17s0000g07630	DEL1 (DP-E2F-like 1)	-14.8
VIT_13s0067g03250	CENP-E like kinetochore protein	-14.9

VIT_10s0116g01610	monocopper oxidase SKS4 (SKU5 Similar 4)	-15.0
VIT_09s0054g00820	Unknown protein	-15.0
VIT_00s0372g00060	linalool synthase	-15.1
VIT_00s1188g00010	unknown	-15.1
VIT_16s0022g01140	chalcone synthase [Vitis vinifera]	-15.2
VIT_13s0106g00350	lipase GDSL	-15.2
VIT_12s0034g01430	F-box family protein	-15.2
VIT_01s0010g01670	Zinc knuckle	-15.3
VIT_05s0020g02910	STE11 protein kinase homolog NPK1	-15.3
VIT_17s0000g08160	polygalacturonase BURP	-15.4
VIT_19s0014g00090	glucan endo-1.3-beta-glucosidase 4 precursor	-15.4
VIT_08s0056g00210	cyclin-dependent kinase B1;2	-15.4
VIT_10s0003g01820	no hit	-15.4
VIT_07s0005g04770	anthranilate phosphoribosyltransferase	-15.5
VIT_08s0058g00640	patellin-5	-15.5
VIT_04s0008g02690	ELM2 domain-containing protein	-15.5
VIT_07s0005g06000	Pore-forming toxin-like protein Hfr-2	-15.5
VIT_08s0007g06500	Unknown protein	-15.6
VIT_16s0098g01070	calmodulin-binding protein family	-15.6
VIT_03s0063g01990	ternary complex factor MIP1	-15.6
VIT_18s0001g07580	unknown	-15.6
VIT_06s0004g01730	Unknown protein	-15.6
VIT_14s0171g00210	Ankyrin	-15.8
VIT_08s0040g02400	Myosin heavy chain	-15.9
VIT_18s0001g12300	Unknown protein	-15.9
VIT_08s0007g02700	secoisolariciresinol dehydrogenase	-15.9
VIT_10s0003g05480	SEC14 cytosolic factor	-15.9
VIT_15s0048g01730	aurora kinase 3	-16.0
VIT_13s0067g03230	CENP-E like kinetochore protein	-16.0
VIT_18s0001g09920	cyclin delta-3 (CYCD3_1)	-16.0
VIT_14s0066g00600	Unknown protein	-16.1
VIT_03s0038g02140	Auxin transporter protein 2	-16.2
VIT_14s0066g01960	metalloendoproteinase 1 precursor	-16.2
VIT_00s2668g00010	F-box family protein	-16.2
VIT_18s0072g00800	histone H3	-16.2
VIT_10s0116g00080	tetratricopeptide repeat (TPR)-containing SET domain protein	-16.2
VIT_06s0080g00760	Unknown protein	-16.3
VIT_13s0019g01210	Dirigent protein pDIR14	-16.3
VIT_00s0179g00380	beta-ketoacyl-CoA synthase	-16.4
VIT_17s0000g09320	AAA-type ATPase	-16.4
VIT_16s0013g01960	chromosome condensation	-16.4
VIT_12s0134g00500	CYCLIN D6	-16.5
VIT_08s0007g04820	pectate lyase	-16.6
VIT_07s0104g00020	ARMADILLO BTB ARABIDOPSIS PROTEIN 1 ABAP1	-16.7
VIT_08s0007g06800	Chromomethylase	-16.7
VIT_04s0008g03590	phosphorylase	-16.8
VIT_18s0164g00010	Unknown protein	-16.8
VIT_04s0008g02570	REPLICON PROTEIN A (ATRPA2/ROR1/RPA2)	-16.9
VIT_19s0093g00590	Myosin heavy chain-like protein	-16.9
VIT_19s0015g01480	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	-17.0
VIT_16s0039g02440	Unknown protein	-17.0
VIT_06s0004g01080	chromomethylase	-17.0
VIT_00s0508g00040	Unknown protein	-17.4
VIT_01s0026g00630	indole-3-acetate beta-glucosyltransferase	-17.5
VIT_09s0002g02210	ADHESION OF CALYX EDGES (ACE)	-17.5
VIT_08s0007g01600	Unknown protein	-17.5
VIT_12s0057g01030	glucose-6-phosphate 1-dehydrogenase 2. chloroplast precursor	-17.5
VIT_17s0000g06410	MYB transcription factor MIXTA-like 2	-17.5
VIT_19s0014g01370	curculin (mannose-binding) lectin	-17.6
VIT_18s0001g08700	kinesin ZCF125	-17.7
VIT_01s0011g01410	PLATZ transcription factor	-17.8
VIT_12s0028g00940	Electron transfer flavoprotein alpha-subunit precursor (Alpha-ETF)	-18.0
VIT_06s0004g06950	N-acetyltransferase hookless1 HLS1	-18.0
VIT_01s0011g02800	RIC7 (ROP-INTERACTIVE CRIB MOTIF-CONTAINING PROTEIN 7)	-18.1
VIT_12s0028g03360	serine/threonine-protein kinase bub1.checkpoint-associated	-18.1
VIT_06s0004g04440	Osmotin	-18.1
VIT_04s0008g06670	plastocyanin domain-containing protein	-18.1
VIT_14s0083g00120	myb domain protein 91	-18.2
VIT_07s0095g00780	Condensin complex subunit 3 . related	-18.3
VIT_14s0060g02360	histone H2A.4 HTA12	-18.3
VIT_14s0128g00460	kinesin motor protein	-18.4
VIT_07s0255g00040	cell division cycle associated 7 CDCA7	-18.6
VIT_08s0058g00340	Unknown protein	-18.7
VIT_16s0022g00200	structural maintenance of chromosome 2	-18.7
VIT_07s0129g01080	MICROTUBULE END BINDING PROTEIN 1 (EB1)	-18.8
VIT_11s0037g00880	Histone-lysine N-methyltransferase ASHR3	-18.8
VIT_02s0025g01220	high mobility group HMG1/2	-18.8
VIT_19s0090g00500	Unknown protein	-18.9
VIT_01s0010g02430	mitotic spindle checkpoint protein (MAD2)	-19.1
VIT_05s0049g01720	no hit	-19.1
VIT_18s0001g13590	leucine-rich repeat protein kinase	-19.1
VIT_11s0016g04380	Unknown protein	-19.1
VIT_16s0098g00380	wax synthase	-19.2
VIT_12s0057g00720	F-box protein GID2	-19.4
VIT_15s0046g00520	wax synthase	-19.5
VIT_07s0005g01060	Histone H1	-19.6
VIT_05s0062g00260	unknown	-19.6
VIT_07s0255g00020	OBF BINDING PROTEIN 1	-19.7
VIT_16s0050g02160	timeless ATIM	-19.8
VIT_08s0007g03220	unknown	-19.9
VIT_16s0022g01000	chalcone synthase [Vitis vinifera]	-20.0
VIT_04s0008g04200	Unknown protein	-20.0
VIT_18s0001g02350	Laccase	-20.2
VIT_08s0040g02590	kinesin motor protein	-20.4
VIT_03s0088g01160	Unknown protein	-20.4
VIT_16s0022g01820	microtubule associated protein (MAP65/ASE1) PLEIADE	-20.4

VIT_14s0108g00650	chromosome condensation protein	-20.5
VIT_17s0000g05830	Unknown protein	-20.5
VIT_12s0059g01070	chromosome associate protein subunit H	-20.6
VIT_14s0068g00270	hydroxyproline-rich glycoprotein	-20.6
VIT_01s0011g03040	Unknown protein	-20.6
VIT_08s0007g05500	condensin subunit 1	-20.6
VIT_17s0000g07770	LTCOR11	-20.8
VIT_19s0090g00730	no hit	-20.8
VIT_17s0000g08460	carbonic anhydrase. chloroplast precursor	-20.9
VIT_17s0000g06900	DNA helicase SNF2 domain-containing protein	-21.0
VIT_07s0005g03990	Unknown protein	-21.0
VIT_17s0000g04830	DNA Polymerase epsilon. subunit B	-21.4
VIT_08s0007g07790	UVI4 (POLYCHOME. UV-B-INSENSITIVE 4)	-21.5
VIT_18s0001g10540	Dem protein	-21.6
VIT_07s0005g06090	Pore-forming toxin-like protein Hfr-2	-21.6
VIT_18s0001g11930	Thaumatin SCUTL2	-21.8
VIT_06s0004g01710	histone H3	-21.8
VIT_01s0011g02930	DNA-binding protein. chloroplast nucleoid	-22.1
VIT_00s0160g00180	histone H3	-22.2
VIT_13s0067g02280	basic helix-loop-helix (bHLH) family	-22.2
VIT_01s0113g00500	argonaute	-22.2
VIT_00s0184g00040	mini-chromosome maintenance protein MCM6	-22.2
VIT_13s0064g00530	disease resistance protein (NBS-LRR class) RGH1	-22.2
VIT_06s0004g01690	histone H3	-22.4
VIT_04s0023g01610	DDM1 (DECREASED DNA METHYLATION 1)	-22.4
VIT_14s0083g00060	DDT domain-containing protein	-22.7
VIT_18s0001g14320	Unknown protein	-22.7
VIT_19s0085g00690	cyclin-dependent protein kinase CYCB3	-22.7
VIT_19s0015g00490	no hit	-22.8
VIT_19s0015g00610	Unknown protein	-22.9
VIT_07s0129g00230	glucan endo-1.3-beta-glucosidase 7 precursor	-23.3
VIT_12s0142g00280	cell division cyle protein 45	-23.4
VIT_14s0006g01290	myb domain protein 113	-23.5
VIT_06s0004g03470	Unknown protein	-23.6
VIT_10s0003g05570	reticuline oxidase	-23.6
VIT_01s0026g02180	Unknown protein	-23.8
VIT_18s0001g01410	unknown	-23.9
VIT_14s0036g01120	unknown	-23.9
VIT_04s0008g05130	TSO1 (CHINESE FOR 'UGLY')	-24.3
VIT_13s0019g00800	histone H4	-24.3
VIT_16s0022g02190	Chalcone and stilbene synthases	-24.4
VIT_08s0040g01230	auxin transport protein (PIN3)	-24.5
VIT_15s0107g00320	cell division cycle 20. cofactor of APC complex	-24.5
VIT_14s0060g01480	unknown	-24.6
VIT_07s0104g00190	7S globulin precursor. basic	-24.6
VIT_19s0090g00800	F-box protein	-24.6
VIT_18s0072g00270	unknown	-24.8
VIT_04s0008g02770	Syntaxin-related protein KNOLLE	-24.8
VIT_06s0004g05870	tubulin beta-3 chain	-24.9
VIT_04s0008g01970	ER (ERECTA)	-24.9
VIT_13s0067g02530	Unknown protein	-25.1
VIT_19s0027g01850	Unknown protein	-25.1
VIT_03s0091g00390	snakin-1	-25.2
VIT_03s0063g00620	transcriptional factor B3	-25.3
VIT_14s0083g00820	lipase GDSL 7	-25.3
VIT_08s0007g04950	xyloglucan endotransglucosylase/hydrolase 32	-25.3
VIT_13s0067g01420	cyclin 1b (CYC1b)	-25.4
VIT_07s0141g00330	F-box protein (FBL17)	-25.4
VIT_03s0063g01120	Kinesin PHRAGMOPLAST ORIENTING KINESIN 2	-25.5
VIT_07s0104g00500	Unknown protein	-25.5
VIT_06s0009g02610	Unknown protein	-25.8
VIT_11s0065g00460	Potyviral helper component protease-interacting protein 2	-25.8
VIT_18s0001g00040	Unknown protein	-26.1
VIT_00s0313g00010	centromere protein	-26.2
VIT_08s0056g01530	anthranilate phosphoribosyltransferase	-26.6
VIT_18s0001g10070	receptor kinase	-26.6
VIT_05s0049g01390	Unknown protein	-26.7
VIT_11s0016g03640	Rac-like GTP-binding protein ARAC7 (GTPase protein ROP9)	-26.7
VIT_17s0000g03940	ferulate 5-hydroxylase	-27.1
VIT_01s0150g00390	minichromosome maintenance protein 2	-27.4
VIT_01s0026g00030	Unknown protein	-27.4
VIT_16s0050g02500	transcription factor MUTE	-27.5
VIT_14s0066g01470	no hit	-27.9
VIT_04s0008g07040	peroxidase	-27.9
VIT_18s0072g00480	hydroxyproline-rich glycoprotein family protein	-28.0
VIT_18s0001g12580	zinc finger homeobox 33	-28.1
VIT_07s0031g00470	DNA polymerase alpha	-28.2
VIT_09s0002g00570	lipase GDSL 1	-28.3
VIT_01s0011g06430	NADPH-dependent codeinone reductase	-28.3
VIT_09s0002g05680	Unknown protein	-28.5
VIT_00s0267g00030	unknown	-28.5
VIT_00s0527g00030	ARABINOGALACTAN PROTEIN AGP31	-28.6
VIT_00s0340g00090	lateral organ boundaries DOMAIN 6	-28.7
VIT_18s0001g08610	AP2-like AINTEGUMENTA-like	-28.7
VIT_16s0039g02230	UDP-glucose:flavonoid 3-O-glucosyltransferase	-28.7
VIT_03s0180g00040	Cyclin D3_2	-28.7
VIT_01s0010g03490	Caffeoyl-CoA O-methyltransferase	-29.1
VIT_14s0128g00440	TANGLED	-29.3
VIT_07s0005g03090	cyclin-dependent protein kinase CDT1A	-29.4
VIT_13s0019g02710	Rho guanyl-nucleotide exchange factor ROPGEF5	-29.6
VIT_16s0098g01510	histone-lysine N-methyltransferase ATXR6	-29.7
VIT_08s0056g01260	Unknown protein	-30.0
VIT_18s0041g02150	lipase GDSL	-30.0
VIT_16s0022g00060	structural maintenance of chromosome 2	-30.0
VIT_03s0038g02800	cyclin B2;4	-30.1
VIT_04s0008g00530	Serine/threonine-protein kinase Aurora-1	-30.2

VIT_16s0022g01570	3-ketoacyl-CoA synthase	-30.3
VIT_11s0118g00770	Unknown protein	-30.9
VIT_16s0022g01620	Receptor protein kinase	-31.0
VIT_17s0000g09930	Unknown protein	-31.1
VIT_12s0028g03620	Unknown protein	-31.1
VIT_04s0023g03360	Unknown protein	-31.4
VIT_09s0018g01630	TUBBY LIKE PROTEIN 8 TLP8	-31.4
VIT_13s0064g00560	DNA topoisomerase. ATP-hydrolyzing	-31.5
VIT_15s0021g01380	cell division cycle 7	-31.6
VIT_05s0020g03640	Unknown protein	-31.9
VIT_08s0007g03090	kinesin family member 11	-32.3
VIT_16s0022g00180	Structural maintenance of chromosomes SMC2	-32.3
VIT_09s0002g07030	TMM (TOO MANY MOUTHS)	-32.3
VIT_14s0068g02000	ribonucleotide reductase R2	-32.3
VIT_17s0000g04740	transducin family protein / WD-40 repeat	-32.5
VIT_19s0090g01600	kinesin motor protein	-33.1
VIT_00s0616g00030	cell division control protein CDC7	-33.3
VIT_05s0020g01270	unknown	-33.3
VIT_18s0001g07550	kinesin family member 4/7/21/27	-33.8
VIT_04s0044g00560	histone H3	-33.9
VIT_14s0030g00660	shikimate dehydrogenase	-34.0
VIT_06s0009g00820	kinesin motor protein	-34.2
VIT_11s0149g00130	Mini-chromosome maintenance protein MCM5	-34.2
VIT_18s0122g00550	cyclin-dependent kinase B2;1	-34.2
VIT_03s0088g00490	no hit	-34.3
VIT_06s0061g00260	ABC transporter G member 7	-34.7
VIT_19s0015g01230	Unknown protein	-34.8
VIT_11s0052g00750	serine carboxypeptidase 1 precursor	-35.0
VIT_10s0003g04280	BREAST CANCER SUSCEPTIBILITY1	-35.3
VIT_19s0014g02500	dynamin family protein	-35.9
VIT_08s0007g07490	binding	-36.0
VIT_07s0005g01430	PROLIFERA protein	-36.0
VIT_05s0077g01960	chromosoma associate protein subunit H2	-36.1
VIT_08s0007g08800	kinesin motor protein	-36.4
VIT_08s0007g03560	anthocyanin membrane protein 1 (Anm1)	-36.9
VIT_03s0038g00300	minichromosome maintenance protein 4	-37.1
VIT_09s0002g03350	no hit	-37.9
VIT_08s0007g00340	kinesin family member 22	-37.9
VIT_08s0040g00930	Cyclin B-type	-38.0
VIT_14s0060g00450	protein-serine/threonine kinase haspin-related	-38.1
VIT_04s0023g03740	Tetratricopeptide repeat domain male sterility MS5	-38.7
VIT_12s0028g03330	targeting protein	-39.2
VIT_06s0061g00070	ubiquitin-conjugating enzyme E2 C	-39.6
VIT_05s0020g00620	Kinesin PHRAGMOPLAST ORIENTING KINESIN 1	-39.7
VIT_19s0014g00230	PHD finger transcription factor	-40.0
VIT_04s0023g03720	Tetratricopeptide repeat domain male sterility MS5	-40.1
VIT_17s0000g04330	kinesin family member 18/19	-40.7
VIT_08s0040g01130	serine carboxypeptidase II	-41.1
VIT_18s0001g13130	BEL1-LIKE HOMEODOMAIN 10	-41.6
VIT_00s0992g00030	calmodulin-binding	-41.8
VIT_14s0108g00710	chromosome condensation protein	-42.3
VIT_06s0009g00870	Unknown protein	-42.5
VIT_18s0001g03470	Flavonol synthase	-42.6
VIT_19s0090g01360	no hit	-42.6
VIT_12s0028g03190	reticulon family protein	-43.4
VIT_03s0017g02210	unknown	-43.5
VIT_13s0064g01340	histone H3	-44.4
VIT_18s0072g00360	no hit	-45.6
VIT_08s0058g00540	IMK2 (INFLORESCENCE MERISTEM RECEPTOR-LIKE KINASE 2)	-46.3
VIT_01s0127g00800	polyamine oxidase precursor	-46.9
VIT_17s0000g05570	Receptor protein kinase	-47.1
VIT_03s0017g00870	unknown	-48.1
VIT_04s0008g04260	cyclin A2	-48.4
VIT_02s0025g04000	scarecrow transcription factor 29 (SCL29)	-48.8
VIT_00s0179g00340	Histone H2A variant 1 HTA11	-49.2
VIT_02s0154g00600	pectinesterase family	-49.6
VIT_00s1404g00010	calmodulin-binding	-49.7
VIT_07s0104g00960	histone H2AXb HTA3	-50.0
VIT_11s0052g00790	serine carboxypeptidase SCPL9	-50.0
VIT_04s0008g01080	calmodulin-binding region IQD6	-50.6
VIT_03s0097g00700	pathogenesis-related protein 1 precursor (PRP 1)	-51.1
VIT_19s0014g01110	curculin (mannose-binding) lectin	-51.5
VIT_18s0001g02060	Cyclin A1	-51.6
VIT_09s0018g01800	acid phosphatase	-52.4
VIT_18s0001g02400	Laccase	-52.5
VIT_19s0027g01870	Amino acid permease	-53.8
VIT_10s0071g00870	High mobility group B 6	-56.5
VIT_09s0002g05930	Unknown protein	-58.9
VIT_07s0005g01670	no hit	-59.5
VIT_02s0025g04910	GROWTH-REGULATING FACTOR 5	-65.6
VIT_07s0130g00030	proteinase inhibitor se60	-65.7
VIT_12s0142g00240	no hit	-121.4
VIT_10s0116g01580	no hit	-142.1
VIT_04s0079g00690	Glutathione S-transferase 26 GSTF12	-144.0

Supplementary table 4. Differentially expressed genes (> |5| fold) in *VvMYB5b* overexpressing plants compared to the untransformed line.

SEQ_ID	GENE DESCRIPTION	FC
VIT_00s1240g00010	no hit	74.4
VIT_01s0011g05090	Major latex-like protein 34	67.0
VIT_15s0048g01710	alcohol dehydrogenase	64.4
VIT_02s0012g01380	no hit	50.4
VIT_00s0187g00060	no hit	50.4
VIT_18s0166g00010	receptor kinase	48.7
VIT_18s0041g00390	cysteine protease inhibitor	48.5
VIT_18s0157g00170	alliin lyase precursor	45.5
VIT_15s0046g00490	wax synthase	44.5
VIT_09s0054g01410	beta-amyrin synthase	43.5
VIT_18s0041g00370	double strand break repair protein (XRCC4)	43.3
VIT_09s0002g03070	light repressible receptor protein kinase	43.2
VIT_17s0000g05110	CYP78A4	40.7
VIT_07s0031g01370	flavonoid 3-monooxygenase	39.4
VIT_18s0122g01480	CYP87A2	39.3
VIT_02s0087g00930	9-cis-epoxycarotenoid dioxygenase	38.6
VIT_01s0137g00790	no hit	37.0
VIT_06s0004g01420	glycosyl hydrolase family 1 protein	34.7
VIT_12s0142g00040	Glycerol-3-phosphate acyltransferase 1 (AtGPAT1)	34.5
VIT_02s0025g00700	Aluminum-activated malate transporter 9	32.9
VIT_09s0002g05810	Boron transporter-like protein 4	31.7
VIT_08s0007g07670	NAC domain containing protein 47	30.8
VIT_18s0001g03180	nodulin MtN21 family	30.7
VIT_10s0003g04800	dienelactone hydrolase	30.5
VIT_09s0054g01230	beta-Amyrin Synthase	30.2
VIT_00s0194g00180	Unknown protein	30.1
VIT_19s0015g02910	CYP72A1	29.6
VIT_17s0000g00830	nodulin MtN3 family	29.1
VIT_07s0104g00420	endo-1.3;1.4-beta-D-glucanase precursor	28.3
VIT_07s0031g01380	ferulate 5-hydroxylase	27.8
VIT_18s0001g15660	Pathogen-related	26.9
VIT_16s0098g01170	homeobox-leucine zipper protein HB-12	26.5
VIT_13s0074g00090	glycosyl transferase family 47 protein	26.5
VIT_14s0068g00840	RBR1 (RETINOBLASTOMA-RELATED 1)	26.2
VIT_18s0001g00030	CYP87A2	26.2
VIT_08s0040g00920	Glutathione S-transferase 25 GSTU7	25.0
VIT_14s0108g01070	NAC domain containing protein 100	24.1
VIT_06s0004g04210	no hit	23.8
VIT_18s0001g04280	(-)-germacrene D synthase	23.7
VIT_17s0000g09190	octicosapeptide/Phox/Bem1p (PB1) domain-containing protein	23.1
VIT_14s0081g00730	ethylene response factor ERF1	22.4
VIT_06s0061g00180	WD40	22.1
VIT_10s0116g00170	S-locus lectin protein kinase	22.1
VIT_01s0011g04190	no hit	22.0
VIT_04s0023g03230	Auxin-responsive SAUR9	21.5
VIT_04s0044g01150	aminotransferase. class V	21.4
VIT_16s0022g01330	disease resistance protein (NBS-LRR class)	21.4
VIT_18s0001g04480	Germacrene-D synthase	21.2
VIT_04s0008g06210	Nodulin	21.2
VIT_13s0074g00660	ABC transporter G member 22	20.8
VIT_06s0004g05750	copper transporter 1	20.6
VIT_16s0050g01720	receptor serine/threonine kinase PR5K	20.5
VIT_11s0016g05430	Unknown protein	20.4
VIT_16s0148g00250	zinc finger (C3HC4-type RING finger)	20.2
VIT_18s0122g00320	PLATZ transcription factor	20.1
VIT_16s0098g00250	zinc finger (C3HC4-type RING finger)	20.0
VIT_13s0074g00690	ABC transporter G member 22	20.0
VIT_09s0054g01360	cycloartenol synthase	19.9
VIT_01s0026g02710	NAC domain-containing protein 29	19.7
VIT_01s0011g03990	Receptor serine/threonine kinase PR5K-l	19.3
VIT_06s0004g00590	lysine decarboxylase	18.1
VIT_17s0000g08720	RKF2 (RECEPTOR-LIKE SERINE/THREONINE KINASE 2)	17.8
VIT_18s0122g01470	CYP87A2	17.4
VIT_12s0028g01360	PMR5 (POWDERY MILDEW RESISTANT 5)	17.1
VIT_17s0000g05320	IFA binding protein	17.0
VIT_04s0023g00320	auxin efflux carrier protein 8	17.0
VIT_05s0029g01140	sucrose-phosphate synthase	16.9
VIT_09s0002g03020	light repressible receptor protein kinase	16.6
VIT_06s0061g01000	prolylcarboxypeptidase	16.5
VIT_06s0004g04140	myb domain protein 59	16.5
VIT_05s0049g01100	Glutathione S-transferase 25 GSTU7	16.2
VIT_07s0104g00070	no hit	15.9
VIT_09s0002g07830	leucine-rich repeat protein kinase	15.7
VIT_18s0001g01570	Seed maturation protein PM31	15.7
VIT_16s0050g02740	receptor-like protein kinase	15.5
VIT_18s0001g04550	(-)-germacrene D synthase	15.5
VIT_16s0098g00460	lipase class 3	15.5
VIT_05s0049g01070	Glutathione S-transferase 19 GSTU1	15.3
VIT_02s0025g02570	CYP78A8	15.2
VIT_09s0054g01370	beta-Amyrin Synthase	15.2
VIT_05s0020g00050	no hit	15.1
VIT_19s0015g02420	serine/threonine-protein kinase receptor ARK3	15.0
VIT_13s0074g00700	ABC transporter G member 22	15.0
VIT_14s0068g01160	cytokinin-repressed protein CR9	14.9
VIT_04s0008g04180	arsenite transport protein (ArsB)	14.9
VIT_13s0074g00680	ABC transporter G member 22	14.8
VIT_19s0014g03300	NAC domain containing protein 2	14.8

VIT_17s0000g01750	auxin-independent growth promoter	14.8
VIT_09s0002g03010	light repressible receptor protein kinase	14.7
VIT_09s0054g01290	beta-Amyrin Synthase	14.7
VIT_07s0005g01350	universal stress protein (USP) family protein	14.6
VIT_08s0058g00290	receptor lectin protein kinase	14.6
VIT_01s0010g03930	WRKY DNA-binding protein 75	14.6
VIT_08s0007g00890	Tropinone reductase	14.6
VIT_18s0001g05450	(-)-germacrene D synthase	14.4
VIT_00s0347g00050	Ser/Thr receptor-like kinase1	14.4
VIT_02s0025g03310	arsenite transport protein (ArsB)	14.4
VIT_08s0007g07730	CYP93A1 2-hydroxyisoflavanone synthase	14.2
VIT_19s0135g00150	CYP72A1	14.1
VIT_14s0108g01250	unknown	14.1
VIT_18s0157g00140	alliin lyase	14.0
VIT_19s0015g02660	CYP72A59	13.9
VIT_18s0001g11580	CYP82A3	13.9
VIT_05s0049g01290	protein phosphatase 2C	13.9
VIT_19s0014g03290	NAC domain containing protein 19	13.9
VIT_19s0014g04080	serine/threonine-protein kinase receptor ARK3	13.8
VIT_03s0017g00660	nematode resistance-like protein	13.7
VIT_05s0049g01090	Glutathione S-transferase 25 GSTU7	13.7
VIT_08s0007g00750	SRG1 (SENESCENCE-RELATED GENE 1)	13.4
VIT_05s0049g01080	Glutathione S-transferase 25 GSTU7	13.3
VIT_03s0097g00460	Geraniol 10-hydroxylase	13.3
VIT_06s0061g00360	UDP-glucoronosyl and UDP-glucosyl transferase	13.2
VIT_19s0014g01940	no hit	13.2
VIT_00s0256g00100	no hit	13.2
VIT_15s0024g00780	no hit	13.2
VIT_16s0039g01210	Ser/Thr receptor-like kinase1	13.1
VIT_17s0000g04400	wall-associated kinase 1 (WAK1)	13.0
VIT_19s0015g02750	CYP72A59	12.9
VIT_18s0089g00370	CARBOXYESTERASE18 CXE18	12.9
VIT_19s0135g00140	CYP72A59	12.8
VIT_05s0049g01120	Glutathione S-transferase 25 GSTU7	12.8
VIT_01s0146g00180	Auxin responsive SAUR protein	12.6
VIT_09s0070g00640	disease resistance protein	12.5
VIT_04s0023g03260	cellulase2	12.4
VIT_12s0057g00870	Cf-2.3	12.4
VIT_04s0008g04940	gibberellin 3beta-hydroxylase3	12.3
VIT_09s0002g00670	Auxin responsive SAUR protein	12.3
VIT_13s0067g01830	steroid 5alpha-reductase	12.3
VIT_19s0015g02900	CYP72A1	12.2
VIT_16s0013g01120	Ethylene-responsive transcription factor ERF105	12.1
VIT_18s0001g06120	cytokinin-O-glucosyltransferase 2	12.1
VIT_18s0166g00060	no hit	12.1
VIT_10s0116g00150	receptor kinase RK20-1	12.0
VIT_00s0187g00050	no hit	11.8
VIT_06s0004g01430	Amygdalin hydrolase isoform AH I	11.8
VIT_10s0071g00450	S-locus lectin protein kinase	11.8
VIT_05s0020g03280	copper amine oxidase	11.8
VIT_01s0011g04700	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	11.8
VIT_19s0014g03730	no hit	11.6
VIT_16s0100g00740	no hit	11.6
VIT_06s0004g07230	indole-3-acetate beta-glucosyltransferase	11.6
VIT_11s0052g01560	MATE efflux family protein	11.5
VIT_04s0044g00270	Monooxygenase (MO3)	11.4
VIT_16s0039g02820	unknown	11.3
VIT_03s0088g00710	pathogenesis-related protein 1 precursor (PRP 1)	11.2
VIT_10s0071g00440	serine/threonine-protein kinase receptor ARK3	11.2
VIT_04s0008g00130	Avr9/Cf-9 rapidly elicited protein 146	11.2
VIT_18s0001g11500	CYP81E1	11.1
VIT_05s0049g01200	S-locus lectin protein kinase	11.1
VIT_00s1682g00020	flavonoid 3'-hydroxylase cytochrome P450	11.1
VIT_02s0025g01750	cellulose synthase CSLG3	11.1
VIT_05s0062g00720	UDP-glucoronosyl/UDP-glucosyl transferase UGT75C1	11.0
VIT_00s0270g00120	alpha-amylase/subtilisin inhibitor	10.9
VIT_00s0294g00070	Ser/Thr receptor-like kinase1	10.9
VIT_01s0010g03210	HcrVf1 protein	10.8
VIT_19s0014g04170	Curculin (mannose-binding) lectin	10.8
VIT_07s0031g00790	steroid nuclear receptor. ligand-binding	10.8
VIT_02s0025g03140	nodulin MtN21 family	10.7
VIT_18s0001g06070	Unknown protein	10.7
VIT_10s0003g00680	glutamate receptor protein	10.7
VIT_18s0001g08430	Branched-chain-amino-acid aminotransferase 2. chloroplast precursor (Atbcat-2)	10.6
VIT_19s0015g02780	CYP72A59	10.6
VIT_07s0104g00270	Isopentenyltransferase 5	10.6
VIT_17s0000g01460	protein kinase AKIN gamma	10.5
VIT_12s0034g01950	legumin	10.5
VIT_03s0180g00290	gibberellin-regulated protein 4 (GASA4)	10.5
VIT_19s0014g00250	bile acid:sodium symporter	10.4
VIT_04s0008g04060	RD22	10.4
VIT_03s0038g04160	DC1 domain-containing protein	10.4
VIT_09s0002g03340	no hit	10.4
VIT_00s0226g00170	no hit	10.4
VIT_18s0001g15220	Unknown protein	10.4
VIT_00s0301g00080	no hit	10.3
VIT_02s0025g00530	unknown	10.3
VIT_09s0018g00670	HcrVf1 protein	10.3
VIT_13s0019g05070	nodulin family protein	10.3
VIT_05s0049g01410	phosphate transporter 1	10.2
VIT_14s0083g00520	proline oxidase	10.2
VIT_02s0025g03120	nodulin MtN21 family	10.1
VIT_00s2840g00010	Phosphoenolpyruvate carboxykinase	10.1
VIT_16s0148g00180	kinase	10.1
VIT_07s0104g01730	HAK5 (High affinity K+ transporter 5)	10.1
VIT_11s0016g01430	zinc finger (C3HC4-type RING finger)	10.1

VIT_15s0024g00770	no hit	10.1
VIT_06s0061g00970	prolylcarboxypeptidase	10.1
VIT_00s0762g00030	S-locus lectin protein kinase	10.1
VIT_11s0052g01250	xyloglucan endotransglucosylase/hydrolase 23	10.1
VIT_12s0034g00030	anthocyanidin 3-O-glucosyltransferase	10.0
VIT_00s1467g00010	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1	10.0
VIT_13s0084g00560	leucine-rich repeat transmembrane protein kinase	9.9
VIT_10s0116g01660	lactoylglutathione lyase	9.9
VIT_18s0001g13210	Lectin jacalin	9.9
VIT_03s0017g01040	cytokinin-O-glucosyltransferase 2	9.9
VIT_09s0002g00540	lipase GDSL 1	9.9
VIT_17s0000g00680	no hit	9.9
VIT_19s0014g04240	S-locus protein kinase	9.8
VIT_12s0034g01890	Cupin region	9.8
VIT_01s0026g01460	Thioredoxin H-type 2 (Trx-H-2)	9.8
VIT_06s0004g03530	NITRATE EXCRETION TRANSPORTER1	9.8
VIT_10s0003g01580	Unknown protein	9.7
VIT_10s0003g04080	no hit	9.7
VIT_05s0049g00550	no hit	9.6
VIT_13s0084g00570	leucine-rich repeat transmembrane protein kinase	9.6
VIT_18s0001g13780	CYP71AT2v2	9.6
VIT_18s0001g12450	no hit	9.5
VIT_00s0374g00020	S-locus lectin protein kinase	9.5
VIT_12s0059g02630	ankyrin repeat protein family	9.4
VIT_00s2507g00010	F-box family protein	9.4
VIT_18s0001g08470	myb domain protein 84/RAX3	9.4
VIT_10s0003g00470	isoflavone methyltransferase/ Orcinol O-methyltransferase 1 oomt1	9.4
VIT_09s0054g01430	no hit	9.4
VIT_09s0018g00710	disease resistance family protein	9.4
VIT_03s0063g01770	no hit	9.3
VIT_01s0011g05060	Major latex-like protein 34	9.3
VIT_01s0011g06450	deoxymugineic acid synthase	9.3
VIT_05s0020g01830	UPF0497 family	9.2
VIT_09s0002g04160	thioesterase family	9.2
VIT_16s0050g02730	receptor-like kinase LRK14	9.1
VIT_00s0174g00070	Ankyrin repeat protein	9.1
VIT_18s0089g00410	Sucrose-phosphate synthase 1	9.0
VIT_12s0035g00570	CYP82M1v3	9.0
VIT_00s0258g00040	Ser/Thr receptor-like kinase1	9.0
VIT_08s0007g06310	myb family	9.0
VIT_01s0150g00370	C2 domain-containing protein	9.0
VIT_01s0011g00560	DREPP plasma membrane polypeptide	9.0
VIT_03s0088g00290	Phytosulfokines PSK2	9.0
VIT_16s0148g00090	GASA4	8.9
VIT_18s0166g00050	receptor kinase TRKa	8.9
VIT_09s0002g07750	disease resistance protein	8.9
VIT_06s0004g03200	no hit	8.8
VIT_09s0002g07720	leucine-rich repeat family protein	8.8
VIT_14s0068g01820	Unknown protein	8.8
VIT_18s0001g06090	cytokinin-O-glucosyltransferase 2	8.7
VIT_00s0500g00010	no hit	8.7
VIT_14s0068g01850	no hit	8.7
VIT_18s0075g00330	sucrose-phosphate synthase	8.7
VIT_12s0057g00930	Hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyltransferase	8.7
VIT_06s0004g06030	Calcium/calmodulin-regulated receptor kinase	8.6
VIT_07s0151g00540	indole-3-acetate beta-glucosyltransferase	8.6
VIT_19s0027g00040	CYP72A59	8.5
VIT_02s0012g01160	proton-dependent oligopeptide transport (POT) family protein	8.5
VIT_19s0015g02410	Receptor protein kinase	8.5
VIT_00s0340g00050	endo-1.4-beta-glucanase KORRIGAN (KOR)	8.4
VIT_06s0004g04590	epsin N-terminal homology (ENTH) domain-containing	8.4
VIT_12s0034g01930	globulin-like protein	8.4
VIT_03s0017g01410	no hit	8.4
VIT_00s0414g00040	cellulose synthase CSLE1	8.3
VIT_07s0005g00060	no hit	8.3
VIT_12s0028g01030	unknown	8.3
VIT_05s0077g00900	no hit	8.3
VIT_12s0035g00070	Leucine Rich Repeat receptor-like kinase	8.3
VIT_01s0011g05950	NSL1 (NECROTIC SPOTTED LESIONS 1)	8.2
VIT_07s0129g00830	CYP81D2	8.2
VIT_06s0004g02010	no hit	8.2
VIT_18s0001g04490	germacrene-D synthase	8.2
VIT_08s0058g00410	ferritin 1 (FER1)	8.2
VIT_12s0028g00860	NAC domain-containing protein 42	8.2
VIT_16s0013g01780	Derlin-1	8.1
VIT_16s0148g00040	receptor serine/threonine kinase	8.1
VIT_02s0025g04340	osmotin	8.1
VIT_04s0044g00650	S-receptor kinase	8.1
VIT_10s0003g03490	GA 2-oxidase	8.1
VIT_16s0098g00020	receptor serine/threonine kinase	8.1
VIT_08s0007g02100	Alpha-1.4-glycosyltransferase	8.0
VIT_11s0037g00680	Leucine Rich Repeat receptor-like kinase	8.0
VIT_05s0020g02310	pyruvate.orthophosphate dikinase	8.0
VIT_18s0041g00010	Wall-associated receptor kinase 2	8.0
VIT_12s0057g00210	no hit	8.0
VIT_03s0038g04570	ADP-glucose pyrophosphorylase large subunit 1	8.0
VIT_06s0004g02580	BLH8 (BEL1-LIKE HOMEODOMAIN 8)	7.9
VIT_16s0050g01690	receptor kinase homolog LRK10	7.9
VIT_09s0002g03640	ABC transporter g family pleiotropic drug resistance 7 PDR7	7.9
VIT_01s0011g01790	Galactose-binding like	7.9
VIT_19s0014g04580	S-locus protein kinase	7.9
VIT_06s0004g01030	dirigent protein	7.9
VIT_01s0011g06440	chalcone reductase	7.9
VIT_11s0016g02980	unknown protein	7.8
VIT_12s0034g01910	Glutelin type-A 3	7.8
VIT_18s0001g10150	Ethylene-responsive transcription factor ERF011	7.8

VIT_08s0007g02260	carboxylic ester hydrolase	7.8
VIT_19s0014g04470	S-locus protein kinase	7.8
VIT_03s0063g01780	Extra-large G-protein (XLG1)	7.8
VIT_19s0014g04060	ARK3 (Arabidopsis Receptor Kinase 3)	7.8
VIT_14s0108g00310	Leucine Rich Repeat receptor-like kinase	7.8
VIT_13s0067g03260	pheophorbidase	7.8
VIT_04s0023g02610	epoxide hydrolase 2	7.8
VIT_09s0002g04500	carnitine/acylcarnitine carrier. Mitochondrial	7.8
VIT_00s0258g00010	receptor serine/threonine kinase	7.7
VIT_00s0822g00010	WHITE-BROWN COMPLEX HOMOLOG PROTEIN 11	7.7
VIT_18s0075g00480	TIR-NBS-LRR disease resistance	7.7
VIT_09s0070g00620	leucine-rich repeat family protein	7.7
VIT_18s0089g01140	wall-associated kinase	7.7
VIT_15s0046g01300	no hit	7.6
VIT_18s0001g03880	polcalcin	7.6
VIT_08s0056g00220	Serine/threonine protein phosphatase PP1	7.6
VIT_18s0001g11540	CYPLXXXII	7.6
VIT_04s0044g00680	S-receptor kinase	7.6
VIT_01s0026g01550	Homeodomain leucine zipper protein HB-1	7.6
VIT_09s0002g03320	Unknown protein	7.6
VIT_17s0000g00940	F-box protein	7.6
VIT_07s0005g00870	erg-1	7.6
VIT_09s0054g01330	beta-Amyrin Synthase	7.6
VIT_05s0094g01260	allyl alcohol dehydrogenase	7.5
VIT_00s0131g00320	annexin ANN3	7.5
VIT_16s0022g01900	pentatricopeptide (PPR) repeat	7.5
VIT_14s0108g00340	Leucine Rich Repeat receptor-like kinase	7.4
VIT_16s0050g01940	S-locus lectin protein kinase	7.4
VIT_17s0000g03580	BEE3 (BR ENHANCED EXPRESSION 3)	7.4
VIT_12s0028g03520	S-receptor kinase	7.4
VIT_05s0077g01140	bZIP transcription factor BZIP53	7.4
VIT_05s0020g02200	inosine-uridine preferring nucleoside hydrolase	7.4
VIT_09s0054g01220	cycloartenol synthase	7.4
VIT_09s0054g01520	beta-amyrin synthase	7.4
VIT_16s0148g00260	Ser/Thr receptor-like kinase1	7.4
VIT_14s0030g01890	unknown	7.4
VIT_18s0001g09010	no hit	7.4
VIT_12s0034g01920	no hit	7.3
VIT_02s0012g01040	NAC domain-containing protein 71	7.3
VIT_04s0023g01720	SEC14 cytosolic factor. putative	7.3
VIT_10s0003g00780	Glutamate receptor 3.4	7.2
VIT_16s0115g00220	Myosin-like protein XIA	7.2
VIT_06s0080g00150	subtilisin-like proteinase AIR3	7.2
VIT_07s0191g00010	ABC transporter G member 7	7.2
VIT_11s0118g00040	Receptor Like Protein 9	7.2
VIT_06s0004g05770	heat shock protein 17.4 kDa class I	7.2
VIT_18s0001g11430	flavonoid 3-monooxygenase	7.2
VIT_17s0000g08920	Ribitol dehydrogenase	7.2
VIT_19s0090g01040	no hit	7.1
VIT_08s0040g01720	no hit	7.1
VIT_19s0135g00120	CYP72A59	7.1
VIT_17s0000g01280	WRKY DNA-binding protein 75	7.1
VIT_09s0054g01470	beta-Amyrin Synthase	7.1
VIT_02s0025g02590	Homeobox-leucine zipper protein ATHB-12	7.1
VIT_11s0016g00860	Strictosidine synthase; Quinoprotein amine dehydrogenase. beta chain	7.1
VIT_06s0004g03520	NITRATE EXCRETION TRANSPORTER1	7.1
VIT_11s0016g00520	Auxin-responsive SAUR38	7.1
VIT_08s0040g02600	F-box protein	7.0
VIT_01s0011g06460	deoxymugineic acid synthase	7.0
VIT_08s0007g08750	heat shock transcription factor B3	7.0
VIT_09s0070g00340	Unknown protein	7.0
VIT_17s0000g07790	UDP-glucose:salicylic acid glucosyltransferase	7.0
VIT_00s0291g00050	octicosapeptide/Phox/Bem1p (PB1) domain-containing protein	7.0
VIT_02s0154g00260	Nitrate transporter	7.0
VIT_12s0059g00470	Unknown protein	7.0
VIT_19s0014g04540	S-locus protein kinase	7.0
VIT_08s0032g00700	CXE carboxylesterase	7.0
VIT_06s0009g00530	ABC transporter G member 7	7.0
VIT_00s2485g00010	CRK10 (CYSTEINE-RICH RLK10)	7.0
VIT_00s0625g00020	ABC transporter G member 7	7.0
VIT_08s0007g03870	Phytosulfokines PSK1	6.9
VIT_18s0075g00340	sucrose-phosphate synthase - like protein	6.9
VIT_05s0094g01200	flavonoid 3'-hydroxylase cytochrome P450	6.9
VIT_09s0002g02950	no hit	6.9
VIT_05s0049g00450	nodulin MtN21 family	6.9
VIT_00s2248g00010	rust resistance kinase Lr10	6.9
VIT_10s0042g00590	Leucine-rich repeat receptor-like protein kinase 1	6.9
VIT_02s0025g03010	copper chaperone (CCH)	6.9
VIT_19s0014g04160	ARK3 (Arabidopsis Receptor Kinase 3)	6.8
VIT_06s0004g01990	no hit	6.8
VIT_16s0050g01910	no hit	6.8
VIT_14s0030g00420	inosine-uridine preferring nucleoside hydrolase family protein	6.8
VIT_04s0069g00390	glutamate receptor protein	6.8
VIT_08s0056g00780	MATE efflux family protein	6.8
VIT_00s0420g00040	S-locus lectin protein kinase	6.8
VIT_08s0007g03800	Unknown protein	6.8
VIT_08s0058g00060	Receptor Like Protein 26	6.7
VIT_17s0000g05810	WRKY DNA-binding protein 72	6.7
VIT_07s0031g02950	AMMONIUM TRANSPORTER 2	6.7
VIT_16s0148g00130	receptor serine/threonine kinase	6.7
VIT_19s0014g04450	S-locus protein kinase	6.7
VIT_08s0040g02190	no hit	6.7
VIT_18s0001g08160	no hit	6.7
VIT_19s0014g04790	ORGANIC CATION/CARNITINE TRANSPORTER4	6.7
VIT_06s0061g01070	Oligopeptidase A	6.6
VIT_18s0001g05230	valencene synthase	6.6

VIT_18s0001g15130	WD40 repeat protein	6.6
VIT_19s0014g04180	Curculin (mannose-binding) lectin	6.6
VIT_05s0020g03290	S-receptor kinase	6.6
VIT_09s0002g03100	light repressible receptor protein kinase	6.6
VIT_07s0031g01460	Nuclear transcription factor Y subunit B-3	6.6
VIT_08s0007g04040	flavonoid 3-monooxygenase	6.6
VIT_05s0020g02290	Endonuclease	6.5
VIT_00s0258g00100	receptor serine/threonine kinase	6.5
VIT_03s0017g01550	CRK10 (CYSTEINE-RICH RLK10)	6.5
VIT_06s0004g05760	copper transporter 1	6.4
VIT_19s0014g04630	S-locus protein kinase	6.4
VIT_09s0002g07710	Disease resistance protein	6.4
VIT_16s0050g00140	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	6.4
VIT_16s0100g01200	stilbene synthase	6.4
VIT_06s0004g05800	phosphatidic acid phosphatase / PAP2	6.4
VIT_11s0118g00580	unknown	6.4
VIT_18s0001g15230	unknown	6.4
VIT_19s0014g04090	serine/threonine-protein kinase receptor ARK3	6.4
VIT_02s0241g00100	Unknown protein	6.4
VIT_17s0000g03380	Calmodulin binding protein	6.4
VIT_06s0004g04320	alcohol dehydrogenase 3	6.3
VIT_08s0040g02180	Mlo3	6.3
VIT_04s0023g03470	DNAJ heat shock N-terminal domain-containing protein	6.3
VIT_08s0007g01360	Unknown protein	6.3
VIT_09s0002g01000	Ferredoxin 4Fe-4S. iron-sulfur binding	6.3
VIT_02s0087g00750	Proteinase inhibitor I4. serpin	6.3
VIT_18s0001g14760	lipase 3 (EXL3) family II extracellular	6.3
VIT_09s0002g02080	CYP90D2	6.3
VIT_19s0014g04510	S-locus protein kinase	6.3
VIT_19s0093g00350	Glutathione S-transferase 25 GSTU25	6.3
VIT_18s0001g15700	GLTP3 (GLYCOLIPID TRANSFER PROTEIN 3)	6.2
VIT_18s0001g01280	laccase	6.2
VIT_07s0005g03970	PEPTIDE TRANSPORTER PROTEIN 3	6.2
VIT_14s0006g02630	Receptor-like kinase ARK1AS	6.2
VIT_05s0029g01000	HcrVf3 protein	6.2
VIT_15s0024g01990	zinc finger (C3HC4-type RING finger)	6.2
VIT_08s0040g02200	peroxidase ATP2a	6.2
VIT_10s0042g00960	DNAJ heat shock N-terminal domain-containing protein	6.2
VIT_00s1679g00010	Avr9/Cf-9 rapidly elicited protein 75	6.2
VIT_02s0087g00630	alcohol oxidase	6.2
VIT_16s0148g00280	receptor kinase homolog LRK10	6.2
VIT_16s0148g00240	receptor kinase homolog LRK14	6.1
VIT_18s0001g13770	CYP83C	6.1
VIT_16s0100g01100	Stilbene synthase	6.1
VIT_12s0035g02090	leucine-rich repeat family protein	6.1
VIT_00s0317g00110	esterase/lipase/thioesterase	6.1
VIT_17s0000g08120	no hit	6.1
VIT_05s0062g01160	pectinesterase family	6.1
VIT_18s0001g11420	CYP82C2	6.1
VIT_05s0077g00430	galactinol synthase	6.1
VIT_18s0041g00270	disease resistance RPP13 protein 1	6.1
VIT_16s0100g00830	Stilbene synthase	6.1
VIT_03s0132g00340	Wall-associated receptor kinase-like 10	6.1
VIT_00s0258g00020	receptor serine/threonine kinase	6.1
VIT_01s0011g05930	S-adenosyl-L-methionine:carboxyl methyltransferase	6.1
VIT_12s0035g01240	no hit	6.1
VIT_14s0066g00810	raffinose synthase	6.0
VIT_07s0005g04890	Glutathione S-transferase 25 GSTU7	6.0
VIT_19s0015g00020	ABC transporter C member 9	6.0
VIT_09s0070g00710	isopentenyltransferase	6.0
VIT_10s0003g01160	basic helix-loop-helix (bHLH) family	6.0
VIT_19s0015g00110	CYP71D10	6.0
VIT_03s0017g01940	no hit	6.0
VIT_02s0087g00370	anthranilate N-hydroxycinnamoyl/benzoyltransferase	6.0
VIT_07s0031g01320	TGA-type basic leucine zipper protein TGA1.1	6.0
VIT_13s0067g02880	zinc finger (C3HC4-type RING finger)	6.0
VIT_09s0070g00630	no hit	6.0
VIT_10s0003g00590	Ethylene-responsive transcription factor ERF091	6.0
VIT_00s0258g00050	receptor serine/threonine kinase	6.0
VIT_00s1830g00010	Ser/Thr receptor-like kinase1	6.0
VIT_07s0005g03250	no hit	6.0
VIT_11s0052g00260	Unknown protein	5.9
VIT_04s0008g05750	WRKY DNA-binding protein 18	5.9
VIT_07s0005g00660	Late embryogenis abundant protein 5	5.9
VIT_16s0098g00070	PR5K (PR5-like receptor kinase)	5.9
VIT_15s0046g02550	gibberellin 20-oxidase	5.8
VIT_05s0077g01690	pathogenesis protein 10 [Vitis vinifera]	5.8
VIT_18s0117g00590	laccase	5.8
VIT_04s0023g02900	Ferulate-5-hydroxylase	5.8
VIT_11s0016g01650	ankyrin repeat	5.8
VIT_19s0014g02450	ALF5 (ABERRANT LATERAL ROOT FORMATION 5)	5.8
VIT_17s0000g06110	glutathione S-transferase 9 GSTU9	5.8
VIT_19s0014g04780	no hit	5.8
VIT_09s0002g00700	dormancy/auxin associated protein	5.8
VIT_13s0084g00670	Unknown protein	5.8
VIT_12s0059g02510	zinc finger (B-box type)	5.7
VIT_02s0033g00270	pinoresinol-lariciresinol reductase	5.7
VIT_06s0009g03280	no hit	5.7
VIT_01s0026g00190	armadillo/beta-catenin repeat	5.7
VIT_15s0024g00740	no hit	5.7
VIT_16s0148g00410	receptor serine/threonine kinase	5.7
VIT_08s0040g01060	serine carboxypeptidase S10	5.7
VIT_00s0253g00150	Methyl jasmonate esterase	5.7
VIT_06s0004g03120	MLO-like protein 3	5.7
VIT_06s0004g08330	no hit	5.7
VIT_08s0040g00820	CYP94A1	5.7

VIT_00s0424g00030	receptor serine/threonine kinase	5.7
VIT_18s0001g02100	Unknown protein	5.7
VIT_08s0058g01130	WNK5 (Arabidopsis WNK kinase 5)	5.6
VIT_03s0038g01510	Unknown protein	5.6
VIT_00s0481g00020	diphenol oxidase	5.6
VIT_12s0028g03510	S-locus lectin protein kinase	5.6
VIT_17s0000g05580	isopiperitenol dehydrogenase	5.6
VIT_00s0595g00010	ARK3 (Arabidopsis Receptor Kinase 3)	5.6
VIT_11s0016g01620	no hit	5.6
VIT_18s0001g11570	CYP82C1p	5.6
VIT_16s0148g00030	Receptor-like protein kinase LRK14	5.6
VIT_09s0054g01440	beta-amyrin synthase	5.6
VIT_01s0011g03390	Unknown protein	5.6
VIT_10s0003g00380	homeobox-leucine zipper protein HB52	5.6
VIT_00s0414g00020	cellulose synthase CSLE1	5.6
VIT_19s0014g04530	S-locus receptor kinase	5.6
VIT_15s0048g00530	Auxin-responsive SAUR11	5.6
VIT_14s0068g01300	Serine palmitoyltransferase	5.6
VIT_17s0000g03330	receptor serine/threonine kinase PR5K	5.6
VIT_05s0020g02710	slingshot	5.6
VIT_16s0098g00700	no hit	5.6
VIT_07s0005g01980	glycosyl transferase family 8 protein	5.6
VIT_10s0003g00980	Unknown protein	5.5
VIT_03s0017g01510	N-6 Adenine-specific DNA methylase	5.5
VIT_14s0068g02070	peptidase M48	5.5
VIT_07s0104g01350	integral membrane family protein UPF0497	5.5
VIT_18s0001g11910	1-acyl-sn-glycerol-3-phosphate acyltransferase 4	5.5
VIT_11s0118g00050	EIX receptor	5.5
VIT_00s0454g00020	no hit	5.5
VIT_00s0388g00020	receptor serine/threonine kinase PR5K	5.5
VIT_05s0077g01310	Aldo-keto reductase	5.5
VIT_18s0001g14270	gibberellin-regulated protein 1 (GASA1)	5.5
VIT_16s0013g01110	Ethylene-responsive transcription factor 5	5.5
VIT_19s0015g00240	no hit	5.5
VIT_06s0004g04010	exocyst subunit EXO70 H7	5.5
VIT_18s0041g00020	Wall-associated receptor kinase 2	5.5
VIT_03s0017g01460	no hit	5.5
VIT_13s0019g02660	Unknown protein	5.5
VIT_19s0014g02180	no hit	5.5
VIT_18s0001g08450	branched-chain-amino-acid transaminase ATBCAT-2	5.5
VIT_09s0002g05080	kelch repeat-containing F-box family protein	5.4
VIT_05s0051g00180	Glutathione S-transferase 8 GSTU8	5.4
VIT_13s0067g03510	ARR9 typeA	5.4
VIT_05s0077g01530	pathogenesis protein 10 [Vitis vinifera]	5.4
VIT_18s0001g11130	calmodulin-binding protein AR781	5.4
VIT_07s0031g01890	no hit	5.4
VIT_06s0004g03100	MLO1	5.4
VIT_16s0148g00160	receptor serine/threonine kinase	5.4
VIT_08s0007g06220	no hit	5.4
VIT_10s0003g00090	DRM1 dormancy/auxin associated	5.4
VIT_16s0148g00420	rust resistance kinase Lr10	5.4
VIT_16s0148g00070	receptor-like protein kinase	5.4
VIT_00s0340g00060	endo-1.4-beta-glucanase KORRIGAN (KOR)	5.4
VIT_06s0004g01300	proton-dependent oligopeptide transport (POT) family protein	5.4
VIT_19s0135g00130	CYP72A57	5.4
VIT_17s0000g09470	NITRATE TRANSPORTER3.1	5.3
VIT_06s0004g02830	Unknown protein	5.3
VIT_16s0050g01880	receptor kinase homolog LRK10	5.3
VIT_16s0148g00380	Receptor kinase LRK10	5.3
VIT_14s0006g02850	BEE1 (BR ENHANCED EXPRESSION 1)	5.3
VIT_12s0034g01900	globulin-like protein	5.3
VIT_19s0014g03390	Unknown protein	5.3
VIT_03s0063g01380	Unknown protein	5.3
VIT_02s0012g01010	Leucine-rich repeat	5.2
VIT_11s0016g01530	ankyrin repeat family protein	5.2
VIT_06s0080g00640	no hit	5.2
VIT_19s0014g04040	S-receptor protein kinase	5.2
VIT_12s0057g00220	Unknown protein	5.2
VIT_03s0097g00470	ATHVA22A (Arabidopsis thaliana HVA22 homologue A)	5.2
VIT_05s0020g01420	FPF1 (FLOWERING PROMOTING FACTOR 1)	5.2
VIT_13s0019g00480	zinc finger (C2H2 type) family	5.2
VIT_14s0068g01790	unknown	5.2
VIT_10s0116g00500	Myb CAPRICE CPC	5.2
VIT_00s0388g00090	Ser/Thr receptor-like kinase1	5.2
VIT_13s0156g00550	S-receptor kinase	5.2
VIT_00s0301g00090	NDA2 (ALTERNATIVE NAD(P)H DEHYDROGENASE 2)	5.2
VIT_04s0069g00740	glutamate receptor 2.8	5.2
VIT_02s0025g04330	Thaumatin VVTL1 [Vitis vinifera]	5.2
VIT_05s0077g01150	Beta-1.3-glucanase	5.1
VIT_04s0023g02210	SAM dependent carboxyl methyltransferase Methyltransf_7	5.1
VIT_12s0057g01430	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	5.1
VIT_18s0001g10620	no hit	5.1
VIT_08s0058g00450	substrate carrier. Mitochondrial	5.1
VIT_03s0063g00450	Alpha-amylase	5.1
VIT_10s0116g00900	Unknown protein	5.1
VIT_12s0035g00580	no hit	5.1
VIT_18s0001g14260	no hit	5.1
VIT_01s0011g02520	EXS family protein / ERD1/XPR1/SYG1	5.1
VIT_08s0007g05720	no hit	5.1
VIT_11s0016g01670	ankyrin repeat	5.1
VIT_00s0316g00010	Disease resistance protein	5.1
VIT_19s0015g02540	CYP72A59	5.1
VIT_07s0005g02060	Boron transporter-like protein 1	5.1
VIT_16s0050g02760	receptor serine/threonine kinase	5.1
VIT_16s0148g00170	protein binding / zinc ion binding	5.1
VIT_16s0039g01200	receptor serine/threonine kinase	5.1

VIT_16s0100g00910	stilbene synthase - grape	5.1
VIT_04s0044g00970	unknown protein	5.0
VIT_16s0050g00930	MATE efflux family protein	5.0
VIT_11s0052g00350	receptor kinase TRKe	5.0
VIT_00s0253g00160	Methyl jasmonate esterase	5.0
VIT_16s0050g02100	disease resistance	5.0
VIT_06s0004g08190	Ethylene-responsive transcription factor CYTOKININ RESPONSE FACTOR 4	5.0
VIT_03s0038g03190	no hit	5.0
VIT_02s0025g05110	MATE efflux family protein	5.0
VIT_16s0050g02750	zinc finger (C3HC4-type RING finger)	5.0
VIT_11s0016g04080	Multiprotein-bridging factor 1c MBF1C	5.0
VIT_11s0037g00690	leucine-rich repeat protein kinase	5.0
VIT_02s0012g02220	xyloglucan endotransglucosylase/hydrolase 30	5.0
VIT_18s0089g00630	serine/threonine-protein kinase	5.0
VIT_05s0077g02010	epoxide hydrolase	5.0
VIT_18s0089g00510	Isopentenyltransferase 1	5.0
VIT_00s0485g00010	receptor kinase homolog LRK14	5.0
VIT_07s0005g03220	Ethylene-responsive transcription factor ERF098	5.0
VIT_14s0060g02340	heat shock cognate 70 kDa protein 1	5.0
VIT_12s0035g02080	Unknown protein	-5.0
VIT_10s0092g00170	subtilase	-5.0
VIT_19s0090g01340	no hit	-5.0
VIT_08s0007g02000	glycerol-3-phosphate acyltransferase 8	-5.0
VIT_03s0091g00550	Unknown protein	-5.0
VIT_03s0063g00200	histidinol-phosphatase	-5.0
VIT_07s0141g00720	fimbrin 1	-5.0
VIT_08s0007g02790	Unknown protein	-5.0
VIT_18s0001g10050	LRX1 (LEUCINE-RICH REPEAT/EXTENSIN 1)	-5.0
VIT_12s0059g00570	fasciclin arabinogalactan-protein (FLA7)	-5.0
VIT_12s0057g00700	glucan endo-1.3-beta-glucosidase 3 precursor	-5.0
VIT_08s0040g00490	caspase	-5.0
VIT_12s0059g01320	glucan endo-1.3-beta-glucosidase 7 precursor	-5.0
VIT_14s0006g00250	Cysteine-rich repeat secretory protein 60	-5.1
VIT_17s0000g02700	Alpha-dioxygenase 2	-5.1
VIT_01s0026g00480	Nodulin	-5.1
VIT_14s0068g00370	no hit	-5.1
VIT_18s0001g03610	auxin-independent growth promoter	-5.1
VIT_00s0340g00040	beta-hexosaminidase beta chain precursor	-5.1
VIT_02s0033g00740	no hit	-5.2
VIT_07s0129g00600	Peptidase aspartic	-5.2
VIT_01s0011g04770	armadillo/beta-catenin repeat / U-box domain-containing protein	-5.2
VIT_10s0116g01710	galactosyltransferase family protein	-5.2
VIT_13s0320g00010	lectin	-5.2
VIT_10s0003g03690	beta-1.3-glucanase precursor	-5.2
VIT_07s0005g01180	Unknown protein	-5.2
VIT_08s0007g00670	laccase	-5.2
VIT_05s0051g00050	Myosin-like protein XIB	-5.2
VIT_07s0031g01670	CYP96A10	-5.2
VIT_08s0040g00060	Receptor protein kinase	-5.2
VIT_00s0391g00070	3-deoxy-D-arabino-heptulosonate 7-phosphate synthase	-5.2
VIT_05s0029g00100	beta-D-galactosidase	-5.2
VIT_19s0014g05010	Unknown protein	-5.2
VIT_13s0067g01180	Leaf senescence related	-5.2
VIT_13s0019g02160	laccase	-5.3
VIT_14s0068g00400	hydrolase. alpha/beta fold family	-5.3
VIT_07s0005g01760	Glycerol-3-phosphate acyltransferase 3 (AtGPAT3)	-5.3
VIT_06s0004g07680	nodulin	-5.3
VIT_13s0064g00520	furcatin hydrolase	-5.3
VIT_14s0068g00740	avr9/Cf-9 rapidly elicited protein 146	-5.4
VIT_05s0062g01430	glycosyl hydrolase family 17 protein	-5.4
VIT_02s0033g00260	pinoresinol-lariciresinol reductase	-5.4
VIT_09s0002g01030	subtilisin serine proteinase	-5.4
VIT_05s0020g02280	Unknown protein	-5.4
VIT_09s0002g02220	Protein kinase CDG1	-5.4
VIT_12s0035g00790	Unknown protein	-5.4
VIT_15s0046g02400	glycerol-3-phosphate acyltransferase 8	-5.4
VIT_09s0002g05240	cyclin A2;3	-5.5
VIT_12s0121g00380	xylan 1.4-beta-xylosidase	-5.5
VIT_12s0057g01020	fasciclin-like arabinogalactan protein FLA2	-5.5
VIT_18s0001g05480	Unknown protein	-5.5
VIT_02s0025g03500	Unknown protein	-5.5
VIT_10s0003g01990	RKF1 (RECEPTOR-LIKE KINASE IN FLOWERS 1)	-5.5
VIT_15s0046g02410	Aquaporin TMP-C	-5.5
VIT_00s0218g00220	UDP-glucoronosyl/UDP-glucosyl transferase	-5.5
VIT_03s0038g00830	ARGONAUTE7 AGO7	-5.5
VIT_13s0158g00010	disease resistance protein (NBS class)	-5.5
VIT_14s0036g00820	kinase interacting	-5.5
VIT_09s0002g09090	ser/thr specific protein kinase	-5.5
VIT_13s0019g02500	no hit	-5.5
VIT_08s0007g00210	Unknown protein	-5.5
VIT_17s0000g07470	binding	-5.6
VIT_02s0012g01410	TRN2 (TORNADO 2)	-5.6
VIT_07s0031g00500	subtilase	-5.6
VIT_05s0062g01260	no hit	-5.6
VIT_14s0006g02290	DREB sub A-4 of ERF/AP2 transcription factor	-5.6
VIT_00s0406g00010	beta-hexosaminidase beta chain precursor	-5.6
VIT_00s0229g00120	Ternary complex factor MIP1	-5.6
VIT_08s0007g05000	S-adenosylmethionine synthetase	-5.6
VIT_07s0005g03120	zinc finger (C3HC4-type RING finger)	-5.6
VIT_18s0001g13980	Auxin responsive SAUR protein	-5.6
VIT_19s0090g01280	lipid-binding serum glycoprotein family protein	-5.6
VIT_11s0016g00290	pectinesterase PME3	-5.6
VIT_06s0004g02510	Unknown protein	-5.6
VIT_05s0029g00510	aquaporin PIP PIP1A	-5.7
VIT_07s0005g04410	subtilase	-5.7
VIT_05s0094g00110	Cyclin D3_2	-5.7

VIT_02s0012g00140	NOVEL PLANT SNARE 11	-5.7
VIT_05s0020g03200	Spermine synthase	-5.7
VIT_03s0091g00240	haloacid dehalogenase hydrolase	-5.7
VIT_13s0019g01930	laccase	-5.7
VIT_12s0035g01900	pectinesterase family	-5.7
VIT_02s0025g04550	glucose-methanol-choline (GMC) oxidoreductase	-5.7
VIT_02s0025g00150	PRLI-interacting factor G	-5.8
VIT_16s0098g01740	Unknown protein	-5.8
VIT_18s0001g14660	P-GLYCOPROTEIN 13	-5.8
VIT_17s0053g01040	no hit	-5.8
VIT_05s0049g01860	receptor-like protein kinase	-5.8
VIT_08s0058g01340	leucine-rich repeat transmembrane protein kinase	-5.8
VIT_08s0007g06500	Unknown protein	-5.8
VIT_05s0020g02650	Unknown protein	-5.8
VIT_03s0180g00090	receptor kinase	-5.8
VIT_18s0001g10300	basic helix-loop-helix (bHLH) family	-5.9
VIT_06s0009g03000	Flavonoid 3'.5'-hydroxylase	-5.9
VIT_04s0044g00780	endo-1.4-beta-glucanase	-5.9
VIT_04s0023g03650	Galactose mutarotase-like	-5.9
VIT_05s0062g00610	xyloglucan endotransglucosylase/hydrolase 23	-5.9
VIT_08s0058g00950	curculin-like (mannose-binding) lectin family	-5.9
VIT_06s0061g01010	prolylcarboxypeptidase	-5.9
VIT_19s0085g01110	aspartyl protease	-5.9
VIT_01s0026g00330	NHL repeat-containing protein	-6.0
VIT_16s0022g00690	VASCULAR RELATED NAC-DOMAIN PROTEIN 4	-6.0
VIT_04s0008g01910	ferredoxin-related	-6.0
VIT_00s0583g00030	sphingolipid delta 4 desaturase DES-1	-6.0
VIT_06s0004g04640	Metallothionein 2b	-6.0
VIT_06s0004g08210	Receptor protein kinase	-6.0
VIT_17s0000g06050	ankyrin protein kinase	-6.1
VIT_08s0007g03430	germin	-6.1
VIT_18s0122g00330	Unknown protein	-6.1
VIT_01s0026g01020	binding	-6.1
VIT_01s0026g01420	Wall-associated kinase 4	-6.1
VIT_01s0011g01890	Glutathione S-transferase. C-terminal	-6.1
VIT_11s0016g02180	Amine oxidase	-6.1
VIT_00s1429g00010	kinesin light chain	-6.1
VIT_12s0121g00230	beta-D-xylosidase	-6.1
VIT_06s0004g02520	TELOMERE REPEAT BINDING FACTOR like TRFL6	-6.2
VIT_13s0019g01920	laccase	-6.2
VIT_09s0002g08960	EREBP-4	-6.2
VIT_18s0041g01270	amino acid permease 2	-6.2
VIT_07s0289g00100	acetyl-CoA carboxylase	-6.2
VIT_00s1348g00010	Ternary complex factor MIP1	-6.2
VIT_08s0007g06460	laccase (diphenol oxidase)-like protein	-6.2
VIT_17s0000g01290	RGP1 (REVERSIBLY GLYCOSYLATED POLYPEPTIDE 1)	-6.2
VIT_15s0024g01250	U-box domain-containing protein 15	-6.2
VIT_00s0665g00030	Unknown protein	-6.2
VIT_19s0015g01150	Kinesin 4 motor protein	-6.2
VIT_02s0025g01980	cellulose synthase CSLG3	-6.3
VIT_07s0005g00550	Ribosomal protein S12 (RPS12C) 40S	-6.3
VIT_18s0001g02000	zinc finger (C2H2 type) family	-6.3
VIT_01s0010g02730	chaperone BCS1 mitochondrial	-6.3
VIT_06s0004g06680	ACR4 (ARABIDOPSIS CRINKLY4)	-6.3
VIT_03s0017g00070	glycosyl hydrolase family 17 protein	-6.3
VIT_01s0010g02670	3-oxoacyl-[acyl-carrier-protein] reductase. chloroplast	-6.3
VIT_07s0031g02480	Beta-galactosidase	-6.3
VIT_00s0253g00040	monocopper oxidase SKS17 (SKU5 Similar 17)	-6.3
VIT_15s0045g01440	ECERIFERUM 1 (CER1 protein) Sterol desaturase	-6.3
VIT_18s0001g08550	squalene monooxygenase	-6.3
VIT_09s0002g06990	phosphatidic acid phosphatase / PAP2	-6.4
VIT_11s0103g00180	Kinesin 4 motor protein	-6.4
VIT_18s0001g06220	F-box protein (FBW2)	-6.4
VIT_08s0032g00780	calcium-dependent protein kinase 20 CPK20	-6.4
VIT_02s0087g00280	glycosyl transferase family 1 protein	-6.4
VIT_02s0087g00470	anthraniloyal-CoA: methanol anthraniloyal transferase [Vitis labrusca]	-6.4
VIT_03s0063g00770	CARBOXYESTERASE 12; CXE12	-6.4
VIT_18s0001g06690	gibberellin 20 oxidase 2	-6.4
VIT_12s0178g00120	no hit	-6.4
VIT_08s0058g00800	Wound-induced protein WI12	-6.5
VIT_10s0116g01780	cationic peroxidase	-6.5
VIT_18s0001g13230	beta-galactosidase BG1 [Vitis vinifera]	-6.5
VIT_18s0001g09520	CYP81B2v2	-6.5
VIT_18s0001g05180	beta-D-xylosidase	-6.5
VIT_05s0029g00090	beta-galactosidase	-6.5
VIT_05s0020g01110	pectinacetylesterase	-6.5
VIT_08s0007g01680	ceramidase	-6.6
VIT_12s0028g00400	1.3-beta-glucan synthase	-6.6
VIT_05s0094g01030	UDP-glucose:salicylic acid glucosyltransferase	-6.6
VIT_01s0011g03050	Unknown protein	-6.6
VIT_08s0007g03990	Cellulose synthase CSLA09	-6.6
VIT_00s0220g00180	glycosyl hydrolase family 20 protein	-6.6
VIT_15s0048g02680	calmodulin-binding region IQD17	-6.6
VIT_18s0089g01230	fructokinase-2	-6.6
VIT_16s0039g00650	Beta-galactosidase	-6.7
VIT_02s0025g00210	fringe protein	-6.7
VIT_02s0109g00350	carboxyl-terminal proteinase	-6.7
VIT_19s0015g01120	CLAVATA-like kinase	-6.7
VIT_04s0023g02690	Beta-galactosidase	-6.7
VIT_11s0052g01810	protein kinase	-6.8
VIT_15s0045g01520	ECERIFERUM 1 (CER1 protein) Sterol desaturase	-6.8
VIT_14s0066g00100	Unknown protein	-6.8
VIT_11s0016g05840	protease inhibitor/seed storage/lipid transfer protein (LTP)	-6.8
VIT_05s0020g04880	seed specific protein Bn15D14A	-6.8
VIT_07s0205g00180	basic helix-loop-helix (bHLH) family	-6.8
VIT_08s0007g05130	UDP-glucoronosyl/UDP-glucosyl transferase	-6.8

VIT_13s0064g01260	DNA-damage-repair/toleration protein (DRT100)	-6.8
VIT_09s0018g01900	PEPR1 (PEP1 RECEPTOR 1)	-6.8
VIT_05s0124g00140	lipase class 3	-6.9
VIT_14s0066g00020	Unknown protein	-6.9
VIT_14s0108g01270	cyclin-dependent kinase CDC2C	-6.9
VIT_05s0102g00780	Unknown protein	-6.9
VIT_03s0091g00660	Unknown protein	-6.9
VIT_03s0091g00040	limonoid UDP-glucosyltransferase	-7.0
VIT_06s0061g01230	Cellulose synthase CSLA02	-7.0
VIT_10s0003g01100	Cysteine protease	-7.0
VIT_13s0019g01940	laccase	-7.0
VIT_17s0000g05840	calmodulin binding IQD31 (IQ-domain 31)	-7.0
VIT_08s0007g03520	purple acid phosphatase 22- ATPAP22/PAP22	-7.0
VIT_05s0077g01670	Major cherry allergen Pru av 1.0202	-7.1
VIT_17s0000g01000	Amino acid-binding ACT	-7.1
VIT_15s0048g01130	subtilase	-7.1
VIT_13s0019g01650	alpha-expansin 6 precursor	-7.1
VIT_10s0071g00100	NHL repeat-containing protein	-7.1
VIT_17s0000g02010	atypical receptor kinase MARK	-7.1
VIT_04s0008g06530	formin-2	-7.2
VIT_04s0023g02840	leucine-rich repeat disease resistance protein	-7.2
VIT_18s0001g05840	RNA recognition motif (RRM)-containing protein	-7.3
VIT_12s0028g00310	protein kinase family	-7.3
VIT_00s0583g00020	Unknown protein	-7.3
VIT_14s0171g00100	Blue (type 1) copper domain	-7.3
VIT_13s0067g03050	leucine-rich repeat transmembrane protein kinase	-7.3
VIT_04s0008g07080	chloroplast nucleoid DNA-binding protein	-7.3
VIT_12s0028g03930	retrotransposon protein. unclassified	-7.3
VIT_13s0106g00210	no hit	-7.3
VIT_01s0010g01660	Receptor protein kinase	-7.3
VIT_19s0014g03980	kinase interacting family protein	-7.3
VIT_00s0922g00010	gag-pol polyprotein	-7.3
VIT_15s0048g01210	subtilisin serine endopeptidase (XSP1)	-7.3
VIT_11s0149g00350	chitinase-3 protein 1 precursor	-7.4
VIT_00s0179g00040	Ankyrin	-7.4
VIT_03s0038g03980	Unknown protein	-7.4
VIT_16s0022g00460	no hit	-7.4
VIT_05s0020g00420	Polygalacturonase GH28	-7.5
VIT_18s0122g00620	cinnamoyl-CoA reductase	-7.5
VIT_16s0039g00470	beta-galactosidase	-7.5
VIT_01s0127g00240	retrotransposon protein. unclassified	-7.5
VIT_15s0048g02880	ubiquitin thiolesterase	-7.5
VIT_15s0048g00630	protease inhibitor/seed storage/lipid transfer protein (LTP)	-7.5
VIT_18s0001g09870	Carbohydrate esterase	-7.5
VIT_10s0092g00360	VPS2.2 SNF7	-7.5
VIT_07s0104g01810	Glutathione S-transferase 13 GSTF13	-7.5
VIT_04s0008g05450	phospholipase D alpha 2	-7.6
VIT_08s0007g04380	PAB7 (POLY(A) BINDING PROTEIN 7)	-7.6
VIT_01s0026g00290	no hit	-7.6
VIT_11s0037g01000	clathrin assembly protein 10	-7.6
VIT_13s0047g00200	subtilisin	-7.7
VIT_11s0149g00380	chitinase-3 protein 1 precursor	-7.7
VIT_05s0051g00590	pectate lyase	-7.8
VIT_07s0005g06500	Receptor protein kinase	-7.8
VIT_14s0066g00070	Unknown protein	-7.8
VIT_11s0016g00040	BRI1 (BRASSINOSTEROID INSENSITIVE 1)	-7.8
VIT_00s1373g00020	Unknown protein	-7.9
VIT_08s0040g02340	xylan synthase	-7.9
VIT_04s0023g01120	Galacturonosyltransferase	-7.9
VIT_14s0060g00680	lipase GDSL	-7.9
VIT_03s0091g01050	Nucleobase-ascorbate transporter 4 (NAT4)	-7.9
VIT_16s0039g02600	aspartyl protease	-7.9
VIT_04s0043g00490	no hit	-7.9
VIT_18s0001g00470	monocopper oxidase SKS5 (SKU5 Similar 5)	-8.0
VIT_14s0083g00980	Pollen Ole e 1 allergen and extensin	-8.0
VIT_16s0022g02370	Unknown protein	-8.0
VIT_17s0000g00460	V-type H+-transporting ATPase subunit C	-8.0
VIT_14s0066g00250	lipase GDSL	-8.0
VIT_16s0098g01560	glucan endo-1.3-beta-D-glucosidase	-8.0
VIT_18s0001g10420	Remorin	-8.1
VIT_12s0055g01310	Beta-glucosidase	-8.1
VIT_18s0122g00610	3-hydroxy-3-methylglutaryl-coenzyme A reductase 3	-8.1
VIT_08s0056g01480	cation exchanger. CAX7	-8.1
VIT_07s0005g03390	Unknown protein	-8.1
VIT_14s0030g01430	GCN5 N-acetyltransferase (GNAT)	-8.1
VIT_00s0322g00020	HHP4 (heptahelical protein 4)	-8.2
VIT_16s0050g00830	beta-ketoacyl-CoA synthase	-8.2
VIT_04s0069g00780	Cellulose synthase CSLC05	-8.2
VIT_08s0007g03140	Rac GTPase activating protein 1	-8.2
VIT_12s0028g02240	Thioredoxin TTL3 (TETRATRICOPETIDE-REPEAT THIOREDOXIN-LIKE 3)	-8.2
VIT_06s0004g02690	glycosyl transferase family 2 protein	-8.3
VIT_13s0067g02910	Non-specific lipid-transfer protein	-8.3
VIT_00s0207g00010	anthranilate N-benzoyltransferase protein 1	-8.3
VIT_03s0038g03420	lysine decarboxylase-like protein	-8.4
VIT_03s0038g03660	armadillo/beta-catenin repeat protein / U-box domain-containing	-8.4
VIT_08s0007g05670	unknown	-8.4
VIT_02s0012g01140	Protein kinase	-8.4
VIT_10s0116g01220	beta-galactosidase	-8.5
VIT_11s0103g00470	unknown	-8.5
VIT_01s0010g03540	microtubule associated protein (MAP65-6)	-8.5
VIT_12s0035g01340	Unknown protein	-8.5
VIT_00s0525g00020	squalene monooxygenase	-8.5
VIT_14s0066g00970	anthranilate phosphoribosyltransferase. chloroplast precursor	-8.5
VIT_03s0110g00360	Copper transporter 2	-8.5
VIT_14s0030g01790	Ndr family protein	-8.5
VIT_14s0108g01660	biotin carboxyl carrier protein of acetyl-CoA carboxylase	-8.5

VIT_12s0059g02010	glutamate formiminotransferase	-8.5
VIT_01s0026g00630	indole-3-acetate beta-glucosyltransferase	-8.6
VIT_07s0151g00240	Ankyrin	-8.6
VIT_05s0029g00190	glucan endo-1.3-beta-glucosidase 4 precursor	-8.7
VIT_18s0122g00790	cysteine endopeptidase. papain-type (XCP1)	-8.7
VIT_07s0151g00030	Ankyrin	-8.7
VIT_11s0206g00030	chitinase. class V	-8.7
VIT_13s0073g00150	heat shock protein-related	-8.7
VIT_14s0066g00120	Unknown protein	-8.8
VIT_11s0016g05360	phospholipase D alpha [Vitis vinifera]	-8.8
VIT_16s0115g00490	no hit	-8.8
VIT_06s0004g07700	basic helix-loop-helix (bHLH) family	-8.8
VIT_00s0333g00050	DIR1 (DEFECTIVE IN INDUCED RESISTANCE 1)	-8.8
VIT_10s0003g03590	beta-amyrin synthase	-8.8
VIT_08s0105g00500	unknown protein	-8.9
VIT_03s0091g01090	no hit	-8.9
VIT_18s0001g13380	Papain cysteine proteinase isoform I	-8.9
VIT_08s0007g00660	laccase	-8.9
VIT_19s0085g00900	unknown protein	-8.9
VIT_19s0027g00080	kinesin motor protein	-8.9
VIT_01s0011g05390	invertase/pectin methylesterase inhibitor	-9.0
VIT_08s0007g05160	Flavonoid 3'.5'-hydroxylase	-9.0
VIT_09s0018g01950	Cellulose synthase CSLC05	-9.0
VIT_10s0003g01560	Cellulose synthase CESA8	-9.0
VIT_10s0003g03170	CYP710A1 C-22 sterol desaturase	-9.0
VIT_00s0504g00020	no hit	-9.0
VIT_00s0333g00010	fimbrin 1	-9.0
VIT_00s0945g00010	Unknown protein	-9.0
VIT_00s0802g00020	Unknown protein	-9.1
VIT_13s0019g02530	subtilisin protease C1	-9.1
VIT_12s0055g00810	cationic peroxidase	-9.3
VIT_09s0002g05700	phototropic-responsive NPH3	-9.3
VIT_05s0051g00690	no hit	-9.4
VIT_14s0068g02010	IMP dehydrogenase/GMP reductase	-9.4
VIT_15s0046g01360	no hit	-9.4
VIT_09s0002g03140	lipase GDSL	-9.4
VIT_00s0441g00020	squalene monooxygenase	-9.5
VIT_13s0084g00820	pepsin A	-9.5
VIT_18s0001g09830	TTL3 (TETRATRICOPETIDE-REPEAT THIOREDOXIN 3); protein binding	-9.5
VIT_18s0001g15510	unknown	-9.5
VIT_07s0031g03220	patellin-1	-9.5
VIT_03s0038g03600	serine/threonine kinase	-9.5
VIT_08s0007g05850	no hit	-9.5
VIT_02s0012g01450	Basic helix-loop-helix BHLH071	-9.6
VIT_07s0104g01800	Glutathione S-transferase 13 GSTF13	-9.6
VIT_08s0007g06910	dirigent pDIR3	-9.6
VIT_14s0066g01850	peroxidase	-9.6
VIT_15s0048g03000	GTPase RABA3	-9.6
VIT_01s0010g01200	cell elongation protein / DWARF1 / DIMINUTO (DIM)	-9.7
VIT_05s0020g04190	PINHEAD (Protein ZWILLE)	-9.7
VIT_02s0154g00080	multi-copper oxidase (SKU5)	-9.8
VIT_08s0040g02350	xylan synthase	-9.8
VIT_17s0000g09700	Unknown protein	-9.8
VIT_18s0001g05380	TTL1 (TETRATRICOPETIDE-REPEAT THIOREDOXIN-LIKE 1)	-9.8
VIT_19s0090g00900	auxin-responsive protein	-9.9
VIT_10s0003g05030	leucine-rich repeat family protein	-9.9
VIT_08s0007g00410	myb domain protein 91	-9.9
VIT_07s0005g05520	pectate lyase	-10.0
VIT_06s0080g01160	aspartyl protease	-10.0
VIT_10s0092g00150	patatin protein 3	-10.0
VIT_04s0008g01850	Trihelix DNA-binding protein (GT2)	-10.1
VIT_12s0059g00770	monocopper oxidase SKS5 (SKU5 Similar 5)	-10.1
VIT_07s0005g02380	germin-like protein 2 [Vitis vinifera]	-10.1
VIT_04s0044g00990	PGSIP1 (PLANT GLYCOGENIN-LIKE STARCH INITIATION PROTEIN 1)	-10.2
VIT_16s0022g02170	Alpha-L-fucosidase	-10.3
VIT_00s0347g00040	Avr9/Cf-9 rapidly elicited protein 20	-10.3
VIT_13s0064g00430	Steroid sulfotransferase	-10.4
VIT_03s0038g01830	PROLINE-RICH PROTEIN 4	-10.4
VIT_18s0001g12690	Isoflavone reductase protein 4	-10.4
VIT_14s0108g01300	division regulator MinE. Plastid	-10.5
VIT_11s0016g04140	no hit	-10.5
VIT_12s0134g00160	Xyloglucan endotransglycosylase/hydrolase 16	-10.5
VIT_12s0028g02630	Rac-like GTP-binding protein RAC2	-10.7
VIT_01s0011g06010	VACUOLAR IRON TRANSPORTER 1	-10.7
VIT_14s0030g01870	NIMA protein kinase	-10.8
VIT_05s0077g01250	endochitinase A2 precursor	-10.8
VIT_07s0005g02550	IAA30	-10.9
VIT_00s0827g00010	Beta-galactosidase	-10.9
VIT_02s0241g00120	Receptor protein kinase	-10.9
VIT_10s0003g00330	HSL1 (HAESA-LIKE 1)	-10.9
VIT_17s0053g00990	alpha-expansin 1 precursor	-10.9
VIT_13s0067g02280	basic helix-loop-helix (bHLH) family	-11.0
VIT_08s0040g02210	lectin protein kinase	-11.0
VIT_13s0019g02490	subtilisin protease C1	-11.0
VIT_00s0568g00020	gag-pol polyprotein	-11.0
VIT_13s0019g02680	no hit	-11.1
VIT_14s0066g00050	Unknown protein	-11.2
VIT_01s0137g00240	pectate lyase	-11.2
VIT_11s0016g05830	no hit	-11.3
VIT_14s0066g00060	Unknown protein	-11.3
VIT_07s0005g04130	acid phosphatase class B	-11.3
VIT_04s0043g00160	Receptor protein kinase	-11.3
VIT_17s0000g08480	ATMYB66/WER/WER1 (WEREWOLF 1)	-11.3
VIT_01s0011g00590	ACCLIMATION OF PHOTOSYNTHESIS TO ENVIRONMENT	-11.4
VIT_04s0023g02320	jasmonate O-methyltransferase	-11.5
VIT_00s1764g00020	leucine-rich repeat transmembrane protein kinase	-11.6

VIT_15s0021g00040	ECERIFERUM 1 (CER1 protein) Sterol desaturase	-11.6
VIT_16s0050g00100	myosin-related	-11.6
VIT_06s0061g00730	Aquaporin GAMMA-TIP3/TIP1;3	-11.6
VIT_19s0090g00730	no hit	-11.6
VIT_07s0151g00170	Ankyrin	-11.6
VIT_12s0057g00460	auxin-responsive protein	-11.6
VIT_01s0026g00490	Nodulin	-11.7
VIT_09s0002g00450	subtilase	-11.7
VIT_05s0077g02330	transducin protein	-11.7
VIT_06s0004g02880	Receptor-like kinase	-11.7
VIT_18s0001g14910	mannitol dehydrogenase	-11.7
VIT_18s0001g14040	Endo-1.4-beta-glucanase	-11.7
VIT_05s0077g00540	no hit	-11.8
VIT_08s0007g03540	anthocyanin membrane protein 1 (Anm1)	-11.8
VIT_10s0003g03400	no hit	-11.8
VIT_03s0063g01950	no hit	-11.8
VIT_01s0137g00170	b-keto acyl reductase	-11.8
VIT_18s0001g03670	zinc finger (C2H2 type) family	-11.8
VIT_00s2484g00010	VASCULAR RELATED NAC-DOMAIN PROTEIN 4	-11.9
VIT_08s0007g09000	glycerol-3-phosphate dehydrogenase	-12.0
VIT_08s0040g02960	no hit	-12.1
VIT_00s0499g00020	Unknown protein	-12.1
VIT_07s0205g00140	kinesin motor protein	-12.1
VIT_10s0116g01180	unknown protein	-12.1
VIT_02s0025g04910	GROWTH-REGULATING FACTOR 5	-12.1
VIT_04s0008g04230	ABC transporter G member 22	-12.1
VIT_11s0016g01320	myb domain protein 13	-12.2
VIT_08s0007g01850	glycine-rich protein	-12.3
VIT_06s0009g00480	Aluminium-tolerance ALMT1	-12.3
VIT_09s0018g00960	laccase	-12.3
VIT_15s0045g01600	no hit	-12.4
VIT_11s0037g00530	cellulose synthase IRX3	-12.4
VIT_15s0021g00050	ECERIFERUM 1 (CER1 protein) Sterol desaturase	-12.4
VIT_19s0014g03970	Calcium-binding EF-hand	-12.4
VIT_01s0011g03450	Alpha-glucosidase	-12.4
VIT_03s0088g00260	serine carboxypeptidase S10	-12.5
VIT_01s0026g00530	nodulin MtN21 family	-12.5
VIT_06s0004g06570	Calcium/proton exchanger CAX3	-12.7
VIT_07s0141g00740	fimbrin 1	-12.7
VIT_02s0025g00740	pinoresinol forming dirigent protein	-12.9
VIT_10s0116g01670	Prephenate dehydratase with ACT region	-12.9
VIT_07s0031g00130	kinesin protein FRA1 (FRAGILE FIBER 1)	-12.9
VIT_00s0804g00020	Unknown protein	-13.1
VIT_11s0016g05280	peroxidase	-13.2
VIT_01s0011g04080	zinc finger (C3HC4-type RING finger)	-13.2
VIT_02s0012g00660	Calcium-binding EF-hand	-13.2
VIT_03s0038g04000	cysteine endopeptidase. papain-type (XCP1)	-13.3
VIT_13s0047g00340	Ethylene-responsive transcription factor WRINKLED 1	-13.4
VIT_04s0023g02280	S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase	-13.4
VIT_09s0002g00320	pectinesterase PME3	-13.4
VIT_08s0007g01810	ANTR2 (anion transporter 2	-13.4
VIT_11s0016g04540	ABC transporter G member 22	-13.5
VIT_06s0004g02710	drug/metabolite transporter DMT family transporter	-13.5
VIT_01s0011g02550	high mobility group HMG1/2	-13.5
VIT_14s0108g00700	Aquaporin NIP1;2	-13.7
VIT_11s0016g01850	Anthocyanidin-3-glucoside rhamnosyltransferase	-13.8
VIT_10s0071g00860	disease resistance protein	-13.9
VIT_18s0001g00800	tetracycline transporter protein	-13.9
VIT_10s0003g02120	lipase GDSL	-14.0
VIT_05s0049g01990	lipase GDSL	-14.0
VIT_06s0009g00810	periplasmic beta-glucosidase precursor	-14.2
VIT_11s0037g01370	Ankyrin	-14.3
VIT_19s0014g05130	glycosyl transferase family 8 protein	-14.5
VIT_02s0025g01910	cellulose synthase CSLG3	-14.5
VIT_07s0005g01940	pectinesterase family	-14.5
VIT_15s0046g01450	alpha-L-fucosidase	-14.5
VIT_04s0008g00880	HOMEOBOX GENE 1	-14.6
VIT_14s0083g00940	auxin-independent growth promoter	-14.7
VIT_19s0090g01050	endo-beta-1.4-glucanase	-14.8
VIT_11s0037g00910	no hit	-15.0
VIT_04s0023g02870	disease resistance protein Hcr2-5D	-15.0
VIT_06s0004g03760	laccase	-15.0
VIT_16s0022g00670	Vacuolar invertase 1. GIN1	-15.1
VIT_01s0011g03540	lateral organ boundaries protein 41	-15.2
VIT_06s0004g02860	Unknown protein	-15.2
VIT_03s0063g00210	Receptor protein kinase	-15.7
VIT_14s0006g00050	Transposase. IS4	-15.7
VIT_17s0000g05690	isochorismate synthase 1. chloroplast precursor	-15.7
VIT_15s0048g02510	basic helix-loop-helix (bHLH) family	-15.8
VIT_07s0151g00010	no hit	-15.9
VIT_05s0020g00950	aspartyl protease	-15.9
VIT_14s0060g02330	Actin binding FORMIN HOMOLOGY 1	-15.9
VIT_04s0044g00880	WD40	-16.0
VIT_07s0005g04110	Cellulose synthase CESA4	-16.1
VIT_17s0000g02460	WUSCHEL RELATED HOMEOBOX 1	-16.1
VIT_07s0005g00730	pectinesterase family	-16.1
VIT_00s1365g00010	fimbrin 1	-16.2
VIT_18s0001g11300	LYS/HIS TRANSPORTER 7 LHT7	-16.2
VIT_14s0060g01780	Unknown protein	-16.2
VIT_03s0038g02180	glycosyl hydrolase family 10 protein	-16.3
VIT_15s0048g01170	subtilisin serine protease	-16.5
VIT_05s0051g00700	no hit	-16.5
VIT_03s0063g01880	Acyl-CoA synthetase	-16.7
VIT_04s0008g02770	Syntaxin-related protein KNOLLE	-16.9
VIT_00s0194g00280	Cyclin D3_1	-16.9
VIT_14s0068g01620	pollen proteins Ole e I	-17.0

VIT_00s0179g00010	Ankyrin	-17.2
VIT_04s0008g05440	Ethylene-responsive transcription factor SHINE 3	-17.3
VIT_04s0023g02310	S-adenosyl-L-methionine:salicylic acid methyltransferase	-17.3
VIT_02s0025g00730	pinoresinol forming dirigent protein	-17.5
VIT_05s0102g00830	Unknown protein	-17.5
VIT_12s0059g01250	Endo-1.4-beta-glucanase	-17.6
VIT_04s0008g06670	plastocyanin domain-containing protein	-17.7
VIT_02s0025g00260	Polygalacturonase GH28	-17.9
VIT_03s0038g02120	armadillo/beta-catenin repeat	-18.0
VIT_07s0151g00020	Ankyrin	-18.1
VIT_09s0018g01650	WRINKLED1 (AP2/ERBP)	-18.1
VIT_04s0079g00260	Unknown protein	-18.4
VIT_17s0000g02660	myb domain protein 6	-18.4
VIT_05s0051g00680	Unknown protein	-18.6
VIT_07s0031g00540	Rapid ALkalinization Factor RALFL34	-18.6
VIT_05s0020g03930	Sulfate transporter 3.1 (AST12) (AtST1)	-18.7
VIT_01s0127g00870	Polygalacturonase JP630	-18.8
VIT_10s0071g00250	strictosidine synthase	-19.1
VIT_18s0041g02160	lipase GDSL	-19.2
VIT_19s0027g00220	Unknown protein	-19.3
VIT_14s0128g00080	lipase GDSL	-19.4
VIT_14s0066g01960	metalloendoproteinase 1 precursor	-19.4
VIT_14s0060g00350	aspartic protease	-19.5
VIT_00s0261g00040	Unknown protein	-20.2
VIT_08s0058g01210	Non-specific lipid transfer protein LTP1	-20.2
VIT_04s0069g01120	ACYL-ACTIVATING ENZYME 7	-20.4
VIT_04s0008g03950	RD22	-20.4
VIT_11s0052g00570	nodulin MtN21	-20.4
VIT_05s0077g01460	no hit	-20.6
VIT_19s0014g00090	glucan endo-1.3-beta-glucosidase 4 precursor	-20.7
VIT_16s0022g02200	subtilase	-20.9
VIT_01s0127g00850	polygalacturonase BURP	-21.2
VIT_06s0004g03050	fasciclin arabinogalactan-protein (FLA11)	-21.4
VIT_03s0017g01950	pectinesterase family	-21.4
VIT_18s0041g01780	aspartic protease	-21.6
VIT_04s0023g01600	Membrane protein	-22.2
VIT_15s0046g02000	Polygalacturonase GH28	-23.0
VIT_01s0011g03210	aspartyl protease	-23.0
VIT_02s0025g03320	CYP86A2	-23.4
VIT_07s0031g00110	Unknown protein	-23.4
VIT_08s0007g00700	PCS1 (PROMOTION OF CELL SURVIVAL1) peptidase	-23.6
VIT_07s0129g00340	SHOOT GRAVITROPISM 7	-23.9
VIT_10s0116g00520	xyloglucan endotransglucosylase/hydrolase 8	-24.1
VIT_09s0002g04240	Membrane protein	-24.4
VIT_06s0009g03350	phosphatidylglycerol specific phospholipase C	-25.2
VIT_07s0197g00040	LATERAL ORGAN BOUNDARIES domain gene 36	-25.5
VIT_14s0083g01100	alpha-1.4-glucan-protein synthase 1	-25.6
VIT_07s0129g00560	no hit	-26.2
VIT_14s0083g01150	Phytochelatin synthetase	-27.2
VIT_04s0023g01960	laccase (diphenol oxidase)-like protein	-27.2
VIT_06s0004g06090	diphenol oxidase	-27.6
VIT_09s0018g01800	acid phosphatase	-27.6
VIT_06s0009g01640	Receptor protein kinase	-28.0
VIT_03s0038g00150	beta-D-galactosidase	-28.1
VIT_05s0124g00100	no hit	-29.1
VIT_10s0003g00620	lipase GDSL	-29.5
VIT_19s0014g05000	zinc finger (C2H2 type) family	-29.6
VIT_08s0007g02470	aspartyl protease	-30.5
VIT_09s0018g01670	aspartyl protease	-31.2
VIT_06s0004g04050	Laccase	-31.6
VIT_19s0015g00530	fasciclin arabinogalactan-protein (FLA1)	-32.6
VIT_04s0023g02290	S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase	-33.4
VIT_10s0003g05480	SEC14 cytosolic factor	-35.7
VIT_01s0182g00140	PHO1-like protein	-36.4
VIT_14s0068g00260	serine carboxypeptidase S10	-36.6
VIT_15s0048g01750	fasciclin arabinogalactan-protein (FLA8)	-36.7
VIT_10s0003g01050	CHUP1 (CHLOROPLAST UNUSUAL POSITIONING 1)	-37.0
VIT_08s0007g05860	GASA like	-37.5
VIT_04s0043g00560	DD1A protein	-37.6
VIT_14s0060g00330	aspartyl protease	-38.7
VIT_09s0002g03150	lipase GDSL	-39.5
VIT_04s0008g05830	armadillo/beta-catenin repeat	-39.9
VIT_12s0059g01830	no hit	-41.0
VIT_10s0003g02110	lipase GDSL	-41.3
VIT_11s0103g00040	HIGH-AFFINITY K+ TRANSPORTER 1 (HKT1)	-42.1
VIT_06s0004g06730	microsomal omega-3 fatty acid desaturase	-44.6
VIT_04s0008g03550	aquaporin TIP4;1	-50.1
VIT_01s0127g00400	Polygalacturonase GH28	-50.8
VIT_11s0016g00590	invertase/pectin methylesterase inhibitor	-53.8
VIT_01s0026g00570	Bet v I allergen	-59.8
VIT_18s0001g10350	subtilase family protein	-62.2
VIT_11s0103g00190	phospholipase A2 alpha	-68.3
VIT_03s0110g00430	no hit	-71.4
VIT_11s0103g00050	HIGH-AFFINITY K+ TRANSPORTER 1 (HKT1)	-72.5
VIT_16s0022g01970	Anthocyanidin 3-O-glucosyltransferase	-96.0
VIT_03s0110g00370	copper transporter 2	-100.6
VIT_04s0008g05640	protease inhibitor/seed storage/lipid transfer protein (LTP)	-110.6
VIT_06s0080g00760	Unknown protein	-127.7
VIT_18s0001g07340	aspartic proteinase nepenthesin-1 precursor	-179.7

Chapter 4

VvWRKY19 participates to the regulatory network directed by WD40-bHLH-MYB complex

ABSTRACT

In plants, the epidermal cell fate is determined by a ternary complex that is composed of MYB and bHLH transcription factors with a WD40 regulatory protein. The regulatory complex controls many differentiation pathways through the direct activation of the specific set of structural genes. However, in some cases, other intermediate regulators downstream the complex are necessary for the activation of such genes. In Arabidopsis, for example, the WRKY transcription factor, AtTTG2 is under the control of the complex in the regulation of trichome development, PA and mucilage production in seed coat (Johnson *et al.*, 2002; Ishida *et al.*, 2007). Another regulator belonging to the WRKY family is the petunia PH3 that acts downstream and in concert with the regulatory complex in the control of the vacuolar acidification in petal epidermis (Quattrocchio *et al.*, submitted). In grapevine, the WD40-bHLH-MYB complex seems involved in the regulation of the flavonoid pathway, but it is still unknown if it requires the presence of other regulators. In this chapter, we isolated VvWRKY19, the grape ortholog of AtTTG2 and PhPH3. By complementation studies in petunia *ph3* mutants, we demonstrated that VvWRKY19 can fulfil the function of the endogenous PhPH3 in the regulation of vacuolar pH. The silencing of *VvWRKY19* expression by stable transformation of *Vitis vinifera* provided information about its role in grapevine. We suggest that in grapevine VvWRKY19 is an intermediate regulator acting downstream VvMYB5a and VvMYB5b and in concert with the regulatory complex. A microarray analysis on transgenic grapevines silenced for *VvWRKY19* expression provided information on the putative biological processes that this transcription factor regulates.

INTRODUCTION

The plant epidermis is a multifunctional tissue playing important roles in water relations, defence and pollinator attraction. This range of functions is performed by a number of different types of specialized cells, which differentiate from the early undifferentiated epidermis in adaptively significant patterns and frequencies. To achieve cellular diversity and to ensure correct temporal and spatial cellular distribution, both external signals and internal stimuli must be integrated and subsequent regulation of gene expression must be coordinated. Recent reports have provided insight into how a protein complex controls multiple cell fates and has therefore played a key role in the evolution of cellular diversity in the plant epidermis. This transcription complex composed of a WD40, a bHLH and a MYB protein regulates in concert with other regulators the expression of multiple distinct target genes in a range of plant species. Insights into how this complex influences epidermal cell identity were provided by studies of mutants in Arabidopsis, where the complex have been shown to regulate anthocyanin and proanthocyanidin (PA) biosynthesis, mucilage production, trichome and root hair formation. The WD40 protein TRANSPARENT TESTA GLABRA 1 (AtTTG1) induces these processes by forming complexes with different MYB and bHLH proteins (Walker *et al.*, 1999). For example, during trichome development AtTTG1 interacts with the partially redundant bHLHs GLABRA 3 (AtGL3) and ENHANCER OF GLABRA 3 (AtEGL3) and the MYB protein GLABRA 1 (AtGL1) (Kirik *et al.*, 2005), whereas during the suppression of root hair formation in atrichoblasts in the root epidermis, AtTTG1, AtGL3 and AtEGL3 interact with the MYB protein WEREWOLF (AtWER) (Lee and Schiefelbein, 1999) The same bHLH factors with other MYB proteins PRODUCTION OF ANTHOCYANIN PIGMENT 1 AND 2 (AtPAP1 and AtPAP2) induce anthocyanin accumulation in hypocotyls (Borevitz *et al.*, 2000). In seed coat, a similar complex consisting of TTG1, plus the bHLH TRANSPARENT TESTA 8 (AtTT8) and the partially redundant MYB proteins AtMYB5 and TRANSPARENT TESTA 2 (AtTT2) regulates the outer seed coat differentiation (Gonzalez *et al.*, 2009). In particular, AtMYB5 has also pleiotropic control over trichome development, tannin production and mucilage production (Li *et al.*, 2009).

The requirement for a WD40–bHLH–MYB transcription complex has been established also in *Petunia hybrida*, where the processes regulated are the anthocyanin production, vacuolar acidification in petal epidermis and proanthocyanidin synthesis in seeds (Spelt *et al.*, 2002; Zenoni *et al.*, 2011). The WD40 protein ANTHOCYANIN 11 (PhAN11) and the bHLH proteins ANTHOCYANIN 1 (PhAN1) and PhJAF13 interact with distinct MYB factors to activate different pathways. When the complex recruits the MYB protein PhAN2 anthocyanin structural genes are induced (Quattrocchio *et al.*, 1999), while when it is driven by PhPH4, the petunia ortholog of AtMYB5, genes involved in the vacuolar acidification pathways are activated (Quattrocchio *et al.*, 2006).

In addition to the transcription factors belonging to the regulatory complex, other proteins have been identified as additional regulators of the latest stages of these metabolic processes. In Arabidopsis, for example, downstream targets of TTG1-MYB-bHLH complexes include the HD-ZIP transcription factor GLABRA 2 (AtGL2) and the WRKY protein TRANSPARENT TESTA GLABRA 2 (AtTTG2) (Rerie *et al.*, 1994; di Cristina *et al.*, 1996; Johnson *et al.*, 2002; Ishida *et al.*, 2007; Zhao *et al.*, 2008). Both AtGL2 and AtTTG2 have positive roles in trichome outgrowth and branching and in seed coat mucilage production. AtGL2 also regulates root hair patterning while AtTTG2 additionally functions in seed coat PA biosynthesis.

Recently, PhPH3 has been identified as the petunia WRKY transcription factor ortholog to AtTTG2. PhPH3 is part of the regulatory network that leads to the vacuolar acidification of petal epidermis, acting downstream and in concert with the regulatory complex directed by the MYB protein PhPH4 (Quattrocchio *et al.*, submitted). Previous complementation analyses revealed that AtTTG2 and PhPH3 are functionally homologs. In summary, it seems clear that in different plants highly homologous MYB, bHLH and WD40 proteins are responsible for the regulation of several developmental processes. The identification of the intermediate regulators AtTTG2 and PhPH3 showed that also downstream genes are homologous, even if they may play different functions in the native species.

Also in grapevine the presence of the complex WD40–bHLH–MYB was proved (Deluc *et al.*, 2008; Hichri *et al.*, 2010). Each member of the regulatory complex was shown to be ortholog to Arabidopsis and petunia regulators that take part to the complex. As in other plant species, also in grapevine the MYB proteins are the major responsible of the specificity of the complex for the control of the flavonoid pathway branches. VvMYBA1 and VvMYBA2 are responsible for the anthocyanin synthesis (Kobayashi *et al.*, 2002; Kobayashi *et al.*, 2004; Walker *et al.*, 2007), VvMYBF1 for the flavonol branch (Czemmel *et al.*, 2009) and VvMYBPA1 and VvMYBPA2 for the proanthocyanidin pathway (Bogs *et al.*, 2007; Terrier *et al.*, 2009). VvMYB5a and VvMYB5b control the general steps of the flavonoid pathway (Deluc *et al.*, 2006; Deluc *et al.*, 2008). They are orthologs to Arabidopsis MYB5 and petunia PH4 (Deluc *et al.*, 2008).

The identification of the intermediate regulators AtTTG2 and PhPH3 showed that also downstream genes are homologous, even if they play different roles in the respective species. However, no intermediate regulator downstream the transcription complex was previously described in grapevine. In this study we identified VvWRKY19 as the grape transcription factor ortholog to AtTTG2 and functionally homolog to PhPH3. *VvWRKY19* gene encodes a member of the plant-specific WRKY family whose known functions are mainly associated with regulation of pathogen-induced defense, biotic and abiotic stresses (Rushton *et al.*, 2010). Based on our results, we assessed that VvWRKY19 likely acts downstream VvMYB5a and VvMYB5b, but, as recently demonstrated for PhPH3, it can also interact with the complex WD40–bHLH–MYB that may acquire different and/or specific functions. This is in part confirmed by data obtained by microarray analysis on transgenic grapevine silenced for *VvWRKY19*.

MATERIALS AND METHODS

Growth conditions and genetic transformation of *Petunia hybrida*

Petunia plants derived from the collection of Amsterdam University were grown under normal greenhouse conditions. The *ph3* mutated line used for complementation studies is heterozygous for the allele $ph3^{V2068}$ and a stable recessive allele $ph3^{B2267}$ harboring 7bp footprint left by the excision of the transposon *dTpH1* from the third exon of the coding sequence. The genetic transformation of *ph3* mutated line for *35S:VvWRKY19* expression was obtained with leaf disk infiltration by *Agrobacterium tumefaciens* as previously described by van der Meer (1999). Regenerated transgenic shoots were transferred into soil and hardened off in a temperature controlled glasshouse.

Growth conditions and genetic transformation of *Vitis vinifera*

Grapevine (*Vitis vinifera*) tissues of cultivar 'Corvina' were collected from a commercial vineyard during the 2008 to 2009 season in Verona, Italy. Material for nucleic acid extraction was frozen in liquid nitrogen and stored at 80° C.

For the genetic transformation of *Vitis vinifera* 'Shiraz', embyogenic callus was developed from anthers collected during the 2008 and 2009 seasons from Coombe Vineyard, University of Adelaide, Urrbrae, South Australia. Once initiated, the callus was maintained on C1 medium in dark at 28°C and subcultured every 4 weeks. Well-developed material was selected for the transformation and maintained into GS1CA media for 14 days. For inoculation with *Agrobacterium tumefaciens* strain EHA105, the embryogenic callus was collected in a Petri dish and submerged in 3 ml of bacterial suspension for 10 minutes. Bacterial suspension was withdrawn using a transfer pipette and any excess moisture was removed by blotting with sterilized Whatman 3MM filter paper. The agro-infiltrated callus was transferred to a new Petri dish containing 3 pieces of filter paper saturated with liquid modified GS1CA and incubated in the dark at 22°C.

After 48 hours, the embryogenic callus was washed in liquid culture media with timentin (1000 μg/ml) and placed onto GS1CA medium for 9-10 days in the dark at

28°C. To select the transformed material, the callus was then moved into GS1CA supplemented with kanamycin 150 μg/ml; four weeks after the transformation they were subcultured into NN media with the selection antibiotic. Continuing to subculture the material every 4 weeks allowed the germination of GFP positive embryos which were selected at the microscope and collected on MS/2 with 5 μM BAP. When primary shoots emerged, the embryos were transferred firstly in the same media without hormones to help the shoot elongation. Subsequently, plantlets were cut off and transferred into Magenta vessels containing rooting medium and cultured under the same conditions to allow further plant development. Vigorous transgenic plants with well-developed leaves and roots were then transplanted into 7-cm plastic pots containing grape soil mix and acclimated in the greenhouse under the shade for about 3 weeks before transfer to the light.

Bioinformatics

To identify putative WRKY genes in the grape predicted proteome, the PSI-BLAST program from NCBI (Altschul *et al.*, 1997) was queried with a sequence representative for the class I (VIT_01s0011g00220) and one for the class II (VIT_07s0141g00680), with a threshold of e^{-10} and 10 iterations.

The annotation of the protein sequences was performed using HMMscan from the HMMer suite on the Pfam-A database (Eddy, 2008; Finn *et al.*, 2010). In particular, the program HMMer was also employed to create custom HMM profiles with which the proteome database was queried, considering an e-value cut off of $10e^{-2}$. The alignment of 58 grape protein sequences was performed using LINSI from the MAFFT suite (Katoh and Toh, 2008) and was then manually reviewed.

Phylogenetic analysis of grape WRKY proteins was achieved using MrBayes v. 3.12 (Huelsenbeck and Ronquist, 2001). The final tree was calculated discarding as burn-in the first 25% of the runs. Subsequently, a phylogenetic tree comprising Vitis and Arabidopsis WRKY protein sequences was calculated using the PhyML software for

Maximum Likelihood tree inference, using JTT as model for the amino acid substitution.

DNA promoter sequences were screened for plant *cis*-acting regulatory DNA elements manually and by the use of PLACE (http://www.dna.affrc.go.jp/PLACE/ (Higo *et al.*, 1999).

Cloning of *VvWRKY19* cDNA and plasmid constructs

For the heterologous expression of *VvWRKY19* in *Petunia hybrida*, the ORF was amplified by PCR from grapevine (‘Corvina’) cDNA (synthesized from RNA isolated from post-fruit set seed) using Pfu DNA polymerase (Promega) and the primers VvPH3FOR 5’-AAGCAGTCTGGTTATAAGAT-3’ and VvPH3REV 5’-TTCTGTTCCAACTGGACTTT-3’. The generated PCR fragment was purified and directionally cloned into the Gateway entry vector pENTR/D-TOPO (Invitrogen) thanks to the 5'-CACC sequence in the forward primer. After the sequencing, the ORF of interest was transferred into the binary overexpression vector pK7GW2,0 (Laboratory of Plant Systems Biology, PSB; Ghent University, Belgium) by site-specific recombination. The construct was then inserted into *Agrobacterium tumefaciens* EHA105 by electroporation and the transformed strain was then used for petunia transformation.

For *VvWRKY19* overexpression in *Vitis vinifera*, the ORF was amplified by PCR from leaf cDNA of grapevine (‘Shiraz’) with *Herculase II Fusion* DNA Polymerase (Bioline). The forward primer used was WRKYOX FOR 5’-CGGAATTCCAGTGGCACATGTTGTTTTA-3’ and the reverse primer was WRKYOX REV 5’-CGCGGATCCTCATGGTTTCTCTTTATTCG-3’. The generated sequence was purified, sequenced and digested with *BamHI* and *EcoRI* and cloned in the vector pART7 (Gleave, 1992), where *VvWRKY19* is under the control of the CaMV *35S* promoter. The expression cassette was isolated as *NotI* fragment, cloned into the *NotI* site of the binary vector pART27-4a (Gleave, 1992) modified by Walker *et al.* to contain a constitutively-expressed *GFP* gene (unpublished) and transferred into *Agrobacterium tumefaciens* EHA105 by electroporation.

For *VvWRKY19* RNA silencing, a specific sequence at the 3' UTR of the cds was amplified by PCR from leaf cDNA of grapevine ('Shiraz') with *Red Taq* DNA Polymerase (Bioline) in sense orientation (with the primer set: WRKYsi 1 5'-CGCGGATCCACAGATTCTGAGATTCTGGG-3'; WRKYsi 2 5'-CCATCGATTCCACATGCTTGCGCACATTG-3') and antisense orientation (with the primer set: WRKYsi 3 5'-CCG CTCGAGACAGATTCTGAGATTCTGGG-3'; WRKYsi 4 5'-GG GGTACCCCACATGCTTGCGCACATTG-3'). The PCR products were sequenced and then inserted into vector pN6 (Wesley *et al.*, 2001) in sequential cloning steps. In the first cloning step, the *BamHI-ClaI* fragment was inserted into vector pN6 in sense orientation, then the *XhoI-Asp7181* fragment was inserted into the product of this ligation in antisense orientation. This created an inverted repeat of the *VvWRKY19* amplified sequence, separated by an intron and expressed using the *35S* promoter. Sequently, the inverted repeat gene was cloned as a *NotI* fragment into the binary vector pART27-4a. This vector was transferred to the Agrobacterium strain by electroporation.

Transient Transfection Experiments and Dual-Luciferase Assay

As described in Bogs *et al.* (2007), a transient assay was applied using a cell suspension of a Chardonnay petiole callus culture and the dual-luciferase system. Gold particles were coated with a mixture of DNA constructs (1 µg of the respective plasmid) by the method described in Ramsay *et al.* (2003) and used to bombard Chardonnay cells at a helium pressure of 350 kPa within a vacuum of 75 kPa and a distance of 14 cm (Torregrossa L, 2002). For the dual-luciferase assay, each bombardment contained a positive control of 200 ng of the Renilla luciferase plasmid pRluc (Horstmann *et al*., 2004). Cells were harvested 48 h after transfection and lysed by grinding on ice in 150 µl of passive lysis buffer (Promega). After centrifugation of the lysates for 2 min at 500g, measurement of the luciferase activities was performed with a dual-luciferase reporter assay system (Promega) by sequential addition of 25 µl of LARII and Stop & Glo to 10 µl of the lysate supernatant. Light emission was measured with a TD-20/20 luminometer (Turner Design) and the relative luciferase activity was calculated as the ratio between the firefly and the Renilla (control) luciferase activity. All transfection experiments were performed in quadruplicate and each set of promoter experiment was repeated with similar relative ratios to the respective control.

To clone *VvWRKY19* promoter a 1937 bp sequence was isolated by PCR from grapevine genomic DNA (‘Shiraz’) with *Herculase II Fusion* DNA Polymerase (Bioline) using the forward primer WRKY PF1 5’-TACCGGATACTGAAGGCTGT-3’ and the reverse primer WRKY PR1 5’-CAATTGATTAATAGAGTGCTG-3’. The amplified fragment was ligated to pTOPO blunt ended vector (Invitrogen) and sequenced. The vector was digested with *BamHI* and *XhoI* and ligated inside the respective sites into the vector pLUC.

For transient expression of *VvWRKY19*, the 1489 bp ORF was amplified by PCR from leaf cDNA (‘Shiraz’) with *Herculase II Fusion* DNA Polymerase (Bioline). The forward primer used was WRKYOX FOR 5’-CGGAATTCCAGTGGCACATGTTGTTTTA-3’ and the reverse primer was WRKYOX REV 5’-CGCGGATCCTCATGGTTTCTCTTTATTCG-3’. The generated fragment was purified, sequenced, digested with *BamHI* and *EcoRI* and ligated in the vector pART7 (Gleave, 1992). All other promoter luciferase and AtEGL3 construct used in the transfection experiments of this study are described by Bogs *et al.* (2007).

Genomic DNA extraction and Southern Blot

Genomic DNA was extracted from 1 g of leaves (between 1 and 3 cm in length) by the method of Thomas and Scott (1993). For southern blot analysis, genomic DNA (2 µg) was restricted overnight with 20 units of the restriction enzyme *EcoRV* at 37°C. Digests were electrophoresed overnight on 0.7% agarose gels in 1 x TBE buffer. Southern blots were performed using the salt transfer method to Genescreen Plus (Perkin Elmer Life Sciences) and hybridised overnight at 65°C with DNA probes labelled using Rediprime TM (Amersham Biosciences) with ^{32}P-dATP. The filters were washed in 2 x SSC + 1% SDS at 65°C for 10 min and then 0.1 x SSC + 1% SDS at 60°C for 10 min. Filters were exposed to Biomax MS film (Kodak) using Biomax (Kodak) intensifying screens at –8°C for 5 h to 2 days.

Transcriptomic analyses

For gene expression analyses performed using petunia transgenic plants, total RNA from flowers was isolated using in the TRIzol® Reagent (Invitrogen) and following the manufacturer's instructions.

For gene expression analyses performed using grapevine 'Corvina' and 'Shiraz', total RNA was isolated using the Spectrum Plant Total RNA kit (Sigma-Aldrich) according to the manufacturer's instructions. The eluted RNA was further purified with lithium chloride 7.5 M.

For *VvWRKY19* expression analyses conducted using *Petunia hybrida* and *Vitis vinifera* 'Corvina' for expression profile, 1 µg of total RNA was first treated with *DNase I* (Promega) and then reverse transcribed using the I*mprom-IIT _Reverse Transcriptase* (Promega) according to the manufacturer's instructions. The transcriptional profile was analyzed by real time RT-PCR as described by Zenoni *et al.* (2010). using the SYBR Green PCR master mix (Applied Biosystems) and a Mx3000P real time PCR system (Stratagene). Gene-specific primers were designed to anneal in the coding sequence close to the 3′UTR. The forward primer was pH3RT.FOR 5'-AATGTGCGCAAGCATGTGGA -3' to use in combination with the reverse primer pH3RT.REV 5'- CCTGCATATCTGGTTCAGAG-3'. A primer pair was also designed for TC81781 (The Institute for Genomic Research, Release 6.0), encoding an actin protein. Each expression value relative to actin was determined in triplicate. The PCR involved a 50°C hold for 2 min and a 95°C hold for 10 min followed by 40 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 20 s. Nonspecific PCR products were identified by the dissociation curves. Amplification efficiency was calculated from raw data using LingRegPCR software (Ramakers *et al.*, 2003). The relative expression ratio value was calculated for development time points relative to the first sampling time point (post fruit set) according to the Pfaffl equation (Pfaffl, 2001). SE values were calculated according to Pfaffl *et al.* (2002).

For gene expression analysis of transgenic grapevine ‘Shiraz’, first-strand cDNA synthesis was performed with SuperScript III First-Strand Synthesis System (Invitrogen). Transcript levels of *VvWRKY19* were measured by real time RT-PCR, using SYBR green method (ABgene House) on a Rotor-Gene 2000 (version 4.2) real-time cycler (Corbett Research). The thermal cycling conditions were 95°C for 15 min followed by 95°C for 30 s, 58°C for 25 s, and 72°C for 25 s for 35 cycles, followed by a melt cycle from 50°C to 96°C. The primers used were: WRKYsi 1 5’-ACAGATTCTGAGATTCTGGG-3’ and WRKYsi2 5’-CCACATGCTTGCGCACATTG-3’. The gene expression was normalized to *VvUbiquitin1* (TC32075, TIGR database), which transcripts were detected by amplifying a 182-bp product with the primers VvUbiquitin For 5’-GTGGTATTATTGAGCCATCCTT-3’ and VvUbiquitin Rev 5’-AACCTCCAATCCAGTCATCTAC-3’. All samples were measured in triplicate. The difference between the cycle threshold (Ct) of the target gene and the Ct of Ubiquitin, δCt = CtTarget - CtUbiquitin, was used to obtain the normalized expression of the target gene, which corresponds to 22DCt. The Rotor Gene 2000 software (Corbett Research) and the Q-Gene software (Muller *et al.*, 2002) were used to calculate the mean normalized expression of the genes.

Microarray analysis

Total RNA for microarray analysis was isolated from ~100 mg of the ground young leaves tissue using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich). RNA quality and quantity were determined using a Nanodrop 2000 instrument (Thermo Scientific) and a Bioanalyzer Chip RNA 7500 series II (Agilent). The cDNA synthesis, labeling, hybridization and washing reactions were performed according to the NimbleGen Arrays User’s Guide (V 3.2). Each hybridization was carried out on a NimbleGen microarray 090818 Vitis exp HX12 (Roche, NimbleGen Inc.), representing 29,549 predicted genes on the basis of the 12X grapevine V1 gene prediction version (http://srs.ebi.ac.uk/). The chip probe design is available at the following URL: http://ddlab.sci.univr.it/FunctionalGenomics/. The microarray was scanned using a ScanArray 4000XL (Perkin-Elmer) at 532 nm (Cy-3 absorption peak) and GenePix Pro7 software (Molecular Devices) according to the manufacturer’s instructions. Images were analyzed using NimbleScan v2.5 software (Roche), which produces Pair Files containing the raw signal intensity data for each probe and Calls Files with normalized expression data derived from the average of the intensities of the four probes for each gene. The normalized gene expression data were finally converted in log2 values to process data. A Pearson Correlation analysis was carried out to evaluate the robustness of the three biological replicates in each sample. A gene was considered to be expressed if the normalized expression value was higher than the value obtained by averaging the fluorescence of negative control present on the chip for at least two of the three biological replicates. A Significance Analysis of Microarrays (SAM) was implemented using TMeV software (http://www.tm4.org/mev), with a false discovery rate of 2%. Cluster analysis was performed by the k-means method with Pearson's correlation distance (TMeV) referring EV and H gene expression to BV.

Yeast two-hybrid system

For yeast two-hybrid experiments, the coding sequences of *VvWRKY19*, *VvMYB5a*, *VvMYB5b*, *VvMYC1*, *VvWD1* and *VvWD2* previously cloned into the Gateway entry vector pENTR/D-TOPO (Invitrogen) were transferred by site-specific recombination into the vectors pDEST22 (downstream *GAL4 AD* sequence) and pDEST32 (downstream *GAL4 BD* sequence) (Laboratory of Plant Systems Biology, PSB; Ghent University, Belgium). The yeast strain PJ69 (James et al., 1996) harbours *HIS3, ADE2* and *LACz* reporter genes driven by distinct GAL4-responsive promoters. Yeast transformation was performed according to the lithium acetate method (Gietz and Woods, 2002). The vectors pDEST32 (baits) and pDEST22 (preys) were used to transform yeast MATα and MATa strains, respectively. The baits and preys were systematically mated by spotting them on top of each other on a plate with non-selective medium. After overnight incubation, the spots were selected for diploid yeast containing the two plasmids. The plates were incubated for two days at 30°C and then the yeast was transferred to two separate selection media (SD –Trp/-Leu/-Ade and SD –Trp/-Leu/-His/+5mM 3AT). β–galactosidase activity was determined by permealizing the yeasts grown on LT selective dish with chloroform, which was covered with Top-agar and incubated for several hours.

RESULTS

Identification of VvWRKY19 as a transcription factor ortholog of AtTTG2 and PhPH3

The genomic sequence of the near-homozygous PN40024 genotype of *Vitis vinifera* 'Pinot noir' (Jaillon et al., 2007) was searched for WRKY genes, leading to the identification of 52 sequences of this family (Figure 1). This number appears to be consistently smaller in grapevine than in other angiosperm species. In fact the Arabidopsis WRKY family is composed of 72 members (Wu et al., 2005), while 102 genes have been identified in *Oryza sativa* (Ross et al., 2007). The 52 grape WRKY genes identified in this analysis are distributed in almost all chromosomes with the highest concentrations on chromosome 4 and 7.

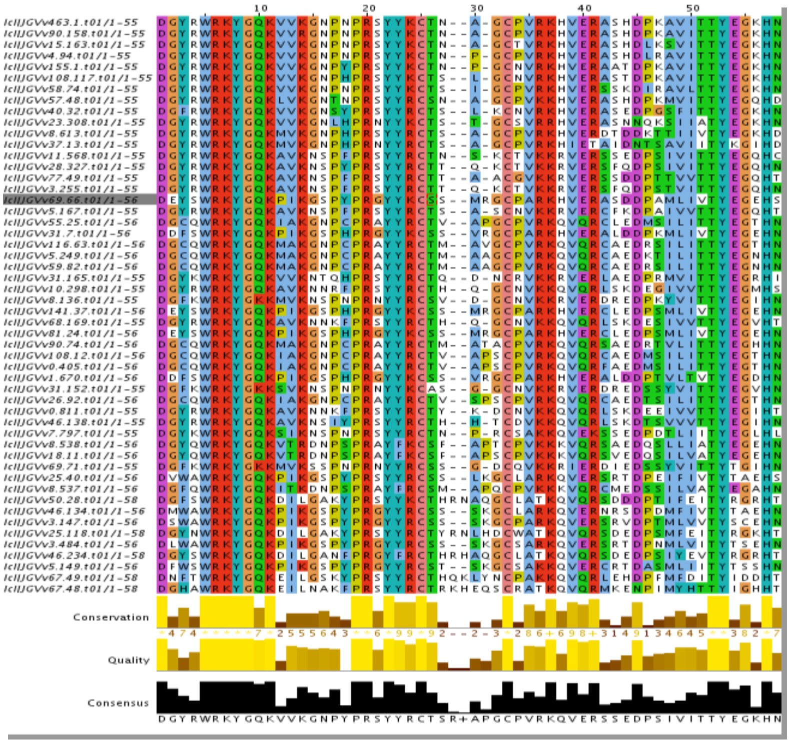

Figure 1. Alignment of 52 vitis sequences belonging to WRKY family used to create the custom HMM profile.

In order to identify the grape candidate homolog to AtTTG2 (AtWRKY44; At2g37260), a phylogenetic tree was built including all the 52 grape and 72 Arabidopsis sequences. As shown in figure 2, the sequence Vv.40.32, previously identified as VvWRKY19 (Gambetta *et al.*, 2010), presented the highest homology with AtTTG2. At the same time, PhPH3 sequence was also used in a BLAST search of the grape genome database. Also in this case, the Vv.40.32 sequence was identified as the most homolog to the query, confirming VvWRKY19 as the best candidate. The full length coding region was amplified from grapevine ‘Corvina’ cDNA of post-fruit set seed. The analysis of the recovered sequence revealed that *VvWRKY19* locus is located on the chromosome 4 as the majority of the WRKY genes in grapevine. It contains a

1434-bp ORF encoding a protein of 477 amino acidic residues with a predicted mass of 52.27 kDa and a calculated pI = 8.84.

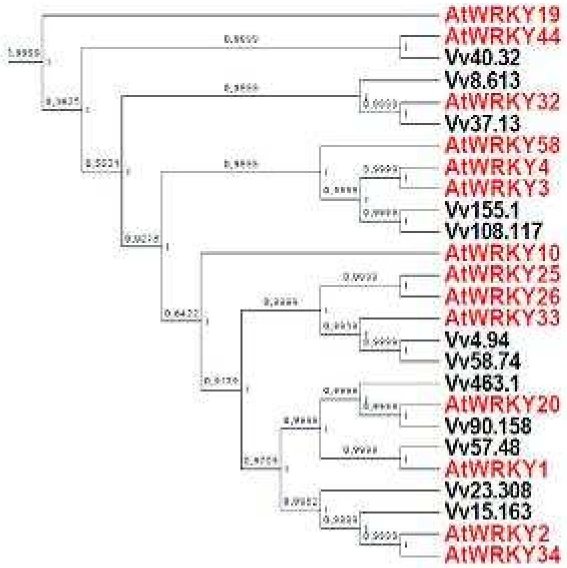

Figure 2. Specific clade containing VvWRKY19 (Vv40.32) and AtTTG2 (AtWRKY44) of the phylogenetic tree of Vitis-Arabidopsis WRKY transcription factors.

The translated sequence isolated from ‘Corvina’ is 99% similar to the predicted one of ‘Pinot noir’. The only different amino acid found in ‘Corvina’ sequence at position 324 is instead conserved in the PhPH3.

Analyses of the primary structure (Figure 3) revealed the presence of two WRKY domains in correspondence to the amino acid 200 and 401, classifying VvWRKY19 in the group I of the family. As for AtTTG2 and PhPH3, the WRKY domains of VvWRKY19 are highly conserved and contain the amino acid sequence WRKYGQK at the N-terminal ends together with the zinc-finger-like motifs. Being the only conserved structural feature, the WRKY domains are assumed to be the DNA-binding domain (Eulgem *et al.*, 2000). The similarity between VvWRKY19 and AtTTG2 and between VvWRKY19 and PhPH3 is 49% and 54% amino acid identity, respectively. As shown in figure 3, *VvWRKY19* gene sequence shares the position of three introns with *AtTTG2* and *PhPH3*. Only for *VvWRKY19* and *PhPH3* is there another fourth intron close to the 3'UTR of the gene.

In conclusion, VvWRKY19 has been identified as the grape ortholog of PhPH3 and AtTTG2.

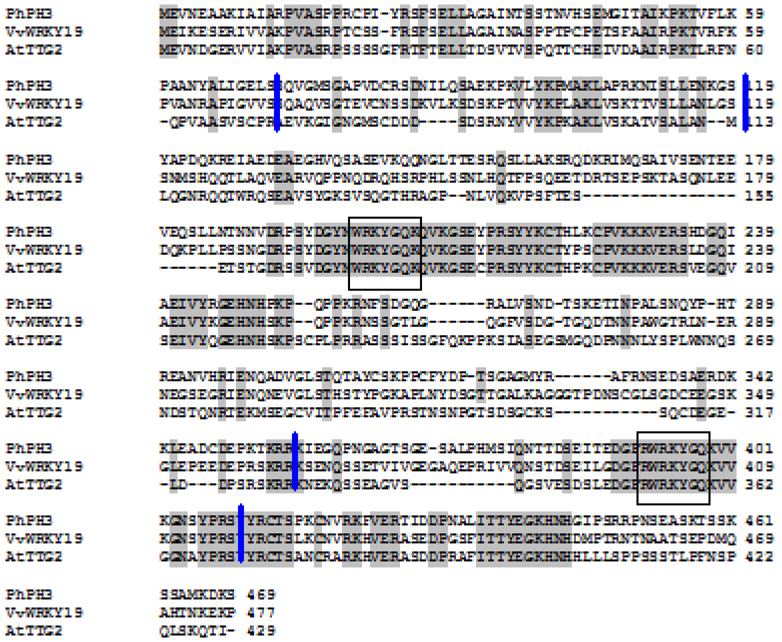

Figure 3. Alignment of the PhPh3, VvWRKY19 and AtTTG2 predicted sequences. Amino acids that are identical are marked in grey. The WRKYGQK motifs are highlighted by open boxes. The blue lines indicate the position of the introns. The first three introns are conserved in all three species, while the last one close to the 3' UTR is present only in petunia and grapevine.

VvWRKY19 expression in grapevine tissues

In order to determine *WRKY19* expression profile of grapevine, we analyzed a global expression map of *Vitis vinifera* 'Corvina' by microarray previously generated (Fasoli, 2012). This transcriptome atlas includes 54 grapevine tissues and organs collected at different stages of development.

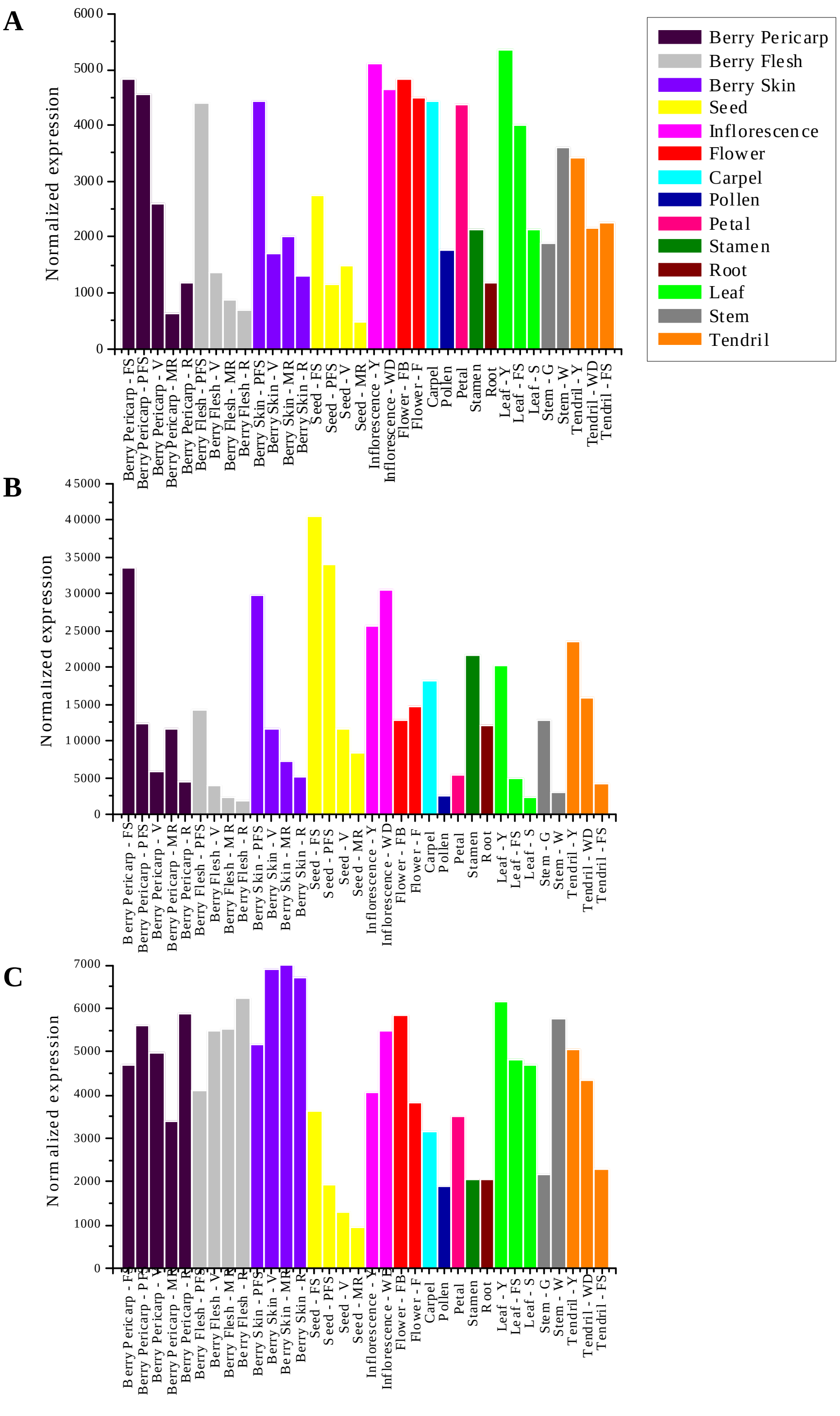
A
B
C
Normalized expression
0
1000
2000
3000
4000
5000
6000
5000
10000
15000
20000
25000
30000
35000
40000
45000
7000
Berry Pericarp
Berry Flesh
Berry Skin
Seed
Inflorescence
Flower
Carpel
Pollen
Petal
Stamen
Root
Leaf
Stem
Tendril
Berry Pericarp - FS
Berry Pericarp - PFS
Berry Pericarp - V
Berry Pericarp - MR
Berry Pericarp - R
Berry Flesh - PFS
Berry Flesh - V
Berry Flesh - MR
Berry Flesh - R
Berry Skin - PFS
Berry Skin - V
Berry Skin - MR
Berry Skin - R
Seed - FS
Seed - PFS
Seed - V
Seed - MR
Inflorescence - Y
Inflorescence - WD
Flower - FB
Flower - F
Carpel
Pollen
Petal
Stamen
Root
Leaf - Y
Leaf - FS
Leaf - S
Stem - G
Stem - W
Tendril - Y
Tendril - WD
Tendril - FS

Figure 4. Expression profiles of *VvWRKY19, VvMYB5a* and *VvMYB5b*. The transcriptomic data were obtained by a global expression map of *Vitis vinifera* 'Corvina' by microarray, performed for 54 grapevine tissues and organs, comprising developmental stages of fruit, reproductive and vegetative organs.

As shown in Figure 4A, *VvWRKY19* expression was detected in all tissues with the highest levels in berry, leaf and many flower-related samples. In berry, the expression appeared high in post-fruit set and then decreased rapidly during the development. The same pattern was observed in flesh, skin, and seed tissues. In leaves, inflorescences and flowers, *VvWRKY19* expression reaches the maximun during the early stages of development. This expression profile was then partially confirmed by investigating *VvWRKY19* transcript levels throughout grape berry development, in leaves, tendrils and inflorescence by real time RT-PCR (Figure 5A).

In summary, expression of *VvWRKY19* gene is not fruit specific but it is expressed early in development and it is clearly down-regulated during grape berry and other organ development.

Is VvWRKY19 an intermediate regulators of flavonoid biosynthetic pathway?

The transcriptomic atlas previously described allowed to compare the expression profiles of *VvWRKY19, VvMYB5a* and *VvMYB5b*. Figure 4 shows that, differently from *VvMYB5b*, *VvWRKY19* and *VvMYB5a* are expressed with a similar pattern in all berry tissues and leaves.

Table 1. Correlation tables for *VvWRKY19* expression considering 54 grapevine tissues and organs at different stages of development (**A**). The same analysis was repeated excluding all berry-related samples (**B**).

A

CORRELATION	SEQ_ID	GENE DESCRIPTION
1.00	**VIT_08s0040g03070**	**PH3**
0.83	VIT_19s0015g02380	ankyrin repeat
0.82	VIT_08s0105g00590	chlorophyll synthetase
0.81	VIT_13s0158g00370	RNA recognition motif (RRM)-containing CCCH
0.81	VIT_08s0056g01120	MATE efflux family protein
0.80	VIT_01s0010g03890	unknown
0.80	VIT_02s0012g00570	pseudo-response regulator 2 (APRR2) (TOC2)
0.79	VIT_11s0037g00070	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase
0.79	VIT_08s0007g08540	Mg-chelatase subunit XANTHA-F
0.79	VIT_08s0007g02490	2-cys peroxiredoxin BAS1. chloroplast precursor
0.78	**VIT_08s0007g07230**	**MYB5a**
0.78	VIT_12s0057g00630	LHCB2.1 (Photosystem II light harvesting complex gene 2.1)
0.78	VIT_17s0000g09370	Elongation factor TuA(EF-TuA)
0.78	VIT_18s0072g00970	DegP protease.
0.78	VIT_02s0012g01050	ABC PROTEIN 6 NON-INTRINSIC
0.78	VIT_07s0031g02560	UVB-resistance protein UVR8
0.78	VIT_08s0040g00390	Magnesium-protoporphyrin IX monomethyl ester [oxidative] cyclase. chloroplast precursor
0.77	VIT_02s0025g03430	GMP synthase
0.77	VIT_08s0040g03010	PIGMENT DEFECTIVE 149
0.76	VIT_08s0056g01070	Protein TRANSPARENT TESTA 12

B

CORRELATION	SEQ_ID	GENE DESCRIPTION
1.00	**VIT_08s0040g03070**	**PH3**
0.82	**VIT_08s0007g07230**	**MYB5a**
0.76	VIT_19s0015g02380	ankyrin repeat
0.75	VIT_11s0016g05350	RabGAP/TBC domain-containing protein
0.75	VIT_04s0044g00550	Carbamoyl phosphate synthetase small subunit
0.74	VIT_02s0241g00040	cyclobutane pyrimidine dimer photolyase
0.74	VIT_08s0056g01120	MATE efflux family protein
0.74	VIT_08s0105g00590	chlorophyll synthetase
0.73	VIT_08s0056g01070	Protein TRANSPARENT TESTA 12
0.73	VIT_11s0016g04850	carboxylic ester hydrolase
0.73	VIT_03s0038g03910	ANTR2 (anion transporter 2)
0.72	VIT_13s0158g00370	RNA recognition motif (RRM)-containing CCCH
0.71	VIT_01s0010g03890	unknown
0.71	VIT_09s0002g00130	VvPH5
0.71	VIT_08s0007g08540	Mg-chelatase subunit XANTHA-F
0.71	VIT_04s0008g01420	HAB2 (Homology to ABI2)
0.71	VIT_15s0046g02560	VVMYCA1
0.71	VIT_06s0004g07710	tRNA pseudouridine synthase
0.71	VIT_02s0012g00570	pseudo-response regulator 2 (APRR2) (TOC2)
0.71	VIT_02s0025g03430	GMP synthase

A co-expression analysis of *VvWRKY19* against all the transcriptome reported in table 1A suggests a correlation between *WRKY19* and *VvMYB5a* expression with a coefficient of 0,79. The same analysis was conducted excluding all berry-related samples, which were over-represented. The coefficient of correlation obtained in this case (Table 1B) confirmed that *WRKY19* and *VvMYB5a* are tightly co-expressed in all the organs excluding the berry. In all transcriptome the gene that mostly correlates with *VvWRKY19* is *VvMYB5a*.

For these reason, we addressed the question if these transcription factors belong to the same regulatory network. The first indication was obtained by microarray analysis on grape hairy roots culture overexpressing *VvMYB5a* (Chapter 3). This analysis revealed that *VvWRKY19* expression was more than 3 fold higher than in the control. Moreover, we investigated *VvWRKY19* expression level in *Vitis vinifera* ‘Shiraz’ silenced for *VvMYB5a / VvMYB5b* by real time RT-PCR. Figure 5B shows that *VvWRKY19* expression is down-regulated in all transgenic plants in comparison to the wild type. All these data indicate that VvWRKY19 could act downstream the regulatory complex driven by VvMYB5a and VvMYB5b.

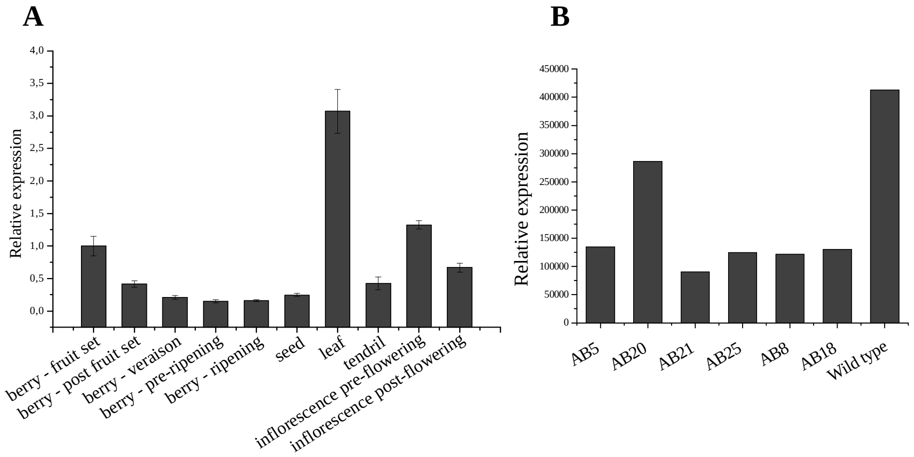

Figure 5. *VvWRKY19* expression analyses by real time RT-PCR.
A) *VvWRKY19* expression profile in grapevine tissues at different stages of development. Data, relative to expression of actin gene, are the mean of three replicates +/- SD.
B) *VvWRKY19* expression in *VvMYB5a / VvMYB5b* silenced grapevines. Data, relative to expression of ubiquitin gene, are the mean of two replicates.

To investigate whether it is a target gene of VvMYB5b and VvMYB5a in the regulatory network, we analyzed the ability of these proteins to activate *VvWRKY19* promoter using a transient expression assay previously described by Bogs *et al.* (2007). The MYB transcription factors VvMYBA1 and VvMYBPA1, anthocyanin- and PA-specific regulators, respectively (Bogs *et al.*, 2007; Walker *et al.*, 2007) were also tested as activators of *VvWRKY19* expression. *VvWRKY19* promoter was fused to the Firefly *luciferase* gene and expressed with the respective transcription factors. All assays included a construct expressing *AtEGL3* which encodes a bHLH protein involved in the regulation of flavonoid pathway in Arabidopsis (Ramsay *et al.*, 2003). Figure 7A shows the activation of *VvWRKY19* expression by all MYBs. The promoter induction detected is 4-5 times that of the negative control, made without the MYB factors. Such values were considered as very low induction of *VvWRKY19* promoter, so the direct activation of *WRKY19* by these MYB transcription factors could not be demonstrated.

Together, these findings suggest that VvWRKY19 acts downstream VvMYB5a and VvMYB5b in the regulatory pathway, but it may be not directly activated by them.

VvWRKY19 interacts with the regulatory complex

In order to investigate interaction properties and specificities of VvWRKY19 with the proteins of the regulatory complex, the yeast two-hybrid system was performed in collaboration with Amsterdam University. We constructed plasmids to express the VvWRKY19, VvMYB5a, VvMYB5b, VvWD1, VvWD2 and VvMYC1 proteins as fusions to either the activation domain of GAL4 (GAL4^{AD}) or to the DNA binding domain (GAL4^{BD}). When expressed (alone) in yeast, VvMYB5a-GAL4^{BD} and VvMYB5b-GAL4^{BD} strongly activated *LACz* reporter gene, whereas GAL4^{AD} did not. In fact, VvMYB5a and VvMYB5b both contain an activation and a DNA binding domain. Because of the auto-activation of VvMYB5a-GAL4^{AD} and VvMYB5b-GAL4^{AD} , fusions to GAL4^{BD} were used to analyze two-hybrid interactions with the other proteins. The interaction between VvMYB5a and VvMYB5b (VvMYB5a-

GAL4^{BD} and VvMYB5b-GAL4^{BD}) and VvMYC1 (VvMYC1-GAL4^{AD}) was previously shown by Hichri *et al.* (2010). On its turn, VvMYC1 (VvMYC1-GAL4^{AD}) was shown to interact with the regulatory protein VvWD1. Similar interactions between a WD40 protein and the bHLH factors have already been described in Arabidopsis (Payne *et al.*, 2000; Zhang *et al.*, 2003; Baudry *et al.*, 2004). Moreover, VvWD1-GAL4^{AD} can interact with VvWRKY19-GAL4^{BD} as shown in figure 6. The interaction between VvWD1 and VvWRKY19 appeared equally strong as that between the WD40 protein PhAN11 with the ortholog PhPH3, because both combinations activate *LACz* gene to a similar extent. Together, these findings indicate that VvWRKY19 may collaborate with the regulatory complex, physically interacting with the WD protein.

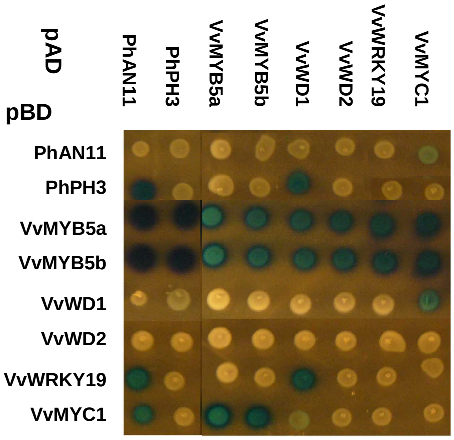

Figure 6. Yeast two-hybrid analysis. Different combinations of plasmids expressing fusion proteins (as indicated at left and at Top of the grid) were co-transformed in yeast, spotted on a plate and assayed for activation of the *LACz* reporter gene (seen as bluing in an X-Gal overlay assay)

To test if VvWRKY19 cooperates with the regulatory complex to control the expression of the flavonoid structural genes, we performed transient assays as previously described by Bogs *et al.* (2007). Therefore, the promoters of the flavonoid genes already known to be activated by VvMYB5a and VvMYB5b (Deluc *et al.*, 2008) were fused to the Firefly *luciferase* gene. The grape chalcone isomerase (*VvCHI*), flavonoid 3'5'-hydroxylase (*VvF3'5'H*), anthocyanidin synthase (*VvANS*) of the general flavonoid pathway and anthocyanidin reductase (*VvANR*) and leucoanthocyanidin reductase (*VvLAR1*) specifically involved in proanthocyanidin synthesis were tested in this experiment. The promoters of these genes were expressed in Chardonnay cells with *VvMYB5a* and *VvMYB5b* alone and in combination with *VvWRKY19*. *VvMYBPA1*, a proanthocyanidin-specific regulator (Bogs *et al.*, 2007) was used as positive control to analyze the expression of the structural genes considered. The Arabidopsis protein EGL3 was used as bHLH co-factor in all the assays. As it was shown in figure 6, VvWRKY19 alone was not able to activate any of these promoters. Moreover, the co-expression of *VvWRKY19* and *VvMYB5a* did not induce any significant increase in promoter activity for *VvCHI, VvF3'5'H* and *VvANS* (Figure 7B, C and D). However, the results of the *VvANR* promoter assays revealed that VvMYB5a activated this promoter 6-fold more when it is expressed in combination with VvWRKY19 (Figure 7F). A slightly increase in the activation capacities was measured for *VvLAR* promoter when activated by VvMYB5b and VvWRKY19 together (Figure 7E). The results of the experiments revealed that VvWRKY19 enhances the activation of proanthocyanidin genes expression activated by VvMYB5a and VvMYB5b. Therefore, one of the VvWRKY19 functions may be the regulation of the PA branch via modifications of the transcriptional activation properties of the WD40–bHLH–MYB regulatory complex.

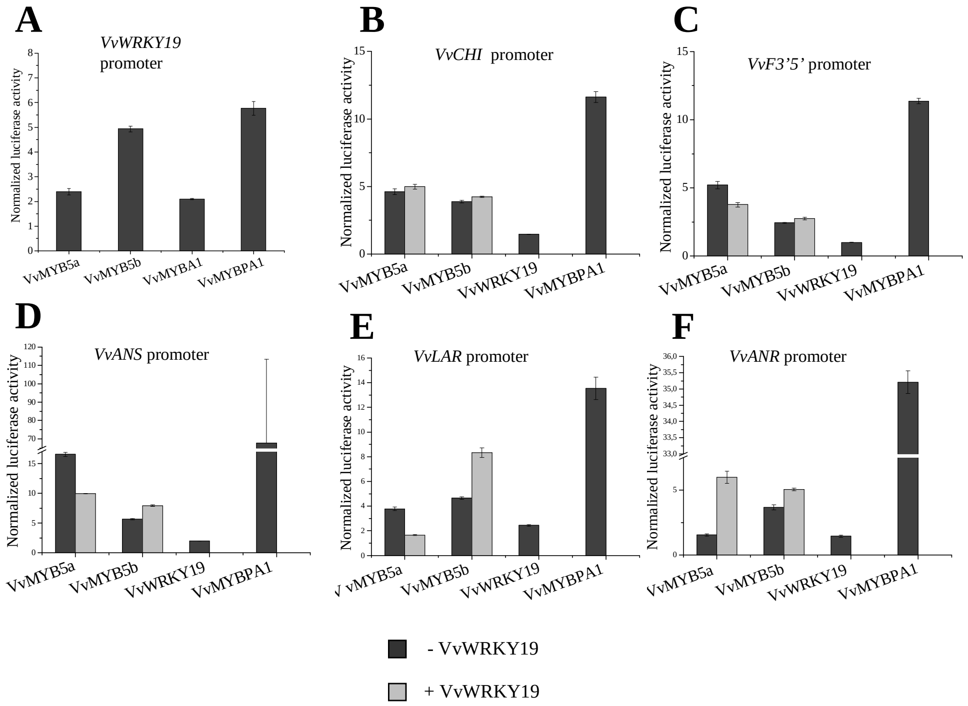

Figure 7. Transient expression analyses.
A) Activation of *VvWRKY19* promoter by VvMYB5a,VvMYB5b, VvMYBPA1 and VvMYBA1.
B – F) Activation of flavonoid gene promoters by VvMYB5a and VvMYB5b in presence or absence of VvWRKY19. The activation induced by VvMYBPA1 was also measured as a comparison. The luciferase activity was normalized against the control rapresented by the respective promoter in the absence of a MYB and WRKY factors. Each transfection contained the *35S::AtEGL3* construct encoding the bHLH protein AtEGL3 (GenBank accession no. NM20235) from Arabidopsis and as internal control the Renilla luciferase plasmid pRLuc. The normalized luciferase activity was calculated as the ratio between the firefly and the Renilla luciferase activity. Each column represents the mean value of four independent experiments with error bars indicating SEs.

Heterologous expression of *VvWRKY19* in petunia *ph3* mutant

To ascertain a putative function of VvWRKY19, we performed complementation analysis in *Petunia hybrida*. *VvWRKY19* cDNA was cloned under the control of the *35S* promoter and used to transform a petunia line mutated in the *PH3* locus. This mutation affects the vacuolar acidification of epidermal cells of petals, resulting in a pale pink pigmentation of the flower. Among 12 PCR positive plants regenerated, we observed a range of phenotypic effects, from the fully complementated evenly red-pigmented corollas to a pale pink corollas similar to the mutant *ph3* flowers (Figure 8).

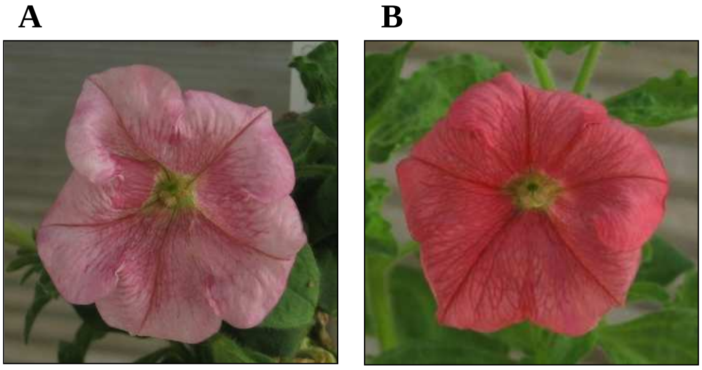

Figure 8. Complementation study of the *VvWRKY19* in petunia *ph3* mutant line.
A) Phenotype of *35S::VvWRKY19* plants in comparison to the untransformed plant (**B**).

These preliminary results show that *VvWRKY19* is able to complement the *ph3* mutation, probably restoring the vacuolar pH and indicate that, besides the sequence similarity, VvWRKY19 and PhPH3 are also functional homologs. However, further experiments are necessary to correlate the complementation of the phenotype with a lower value of the vacuolar pH and to demonstrate that VvWRKY19 regulates the pH structural genes.

Functional analysis of VvWRKY19

In order to understand the regulative functions of VvWRKY19 in grapevine, embryogenic callus of *V. Vinifera* ‘Shiraz’ was transformed with *A. tumefaciens*. Two separate experiments were performed in order to silence and overexpress *VvWRKY19* expression. In addition to the cassette for the overexpression or RNA silencing and the antibiotic selection, the vector used also contains the *GFP* sequence under the control of Arabidopsis *Ubiquitin10* promoter and terminator regions. The presence of the gene reporter allows the additional selection of the transformed embryogenic material during the development of the embryos. Despite three transformation cycles for the overexpression, no transformed embryos were obtained, while 40 PCR positive plantlets were generated for *VvWRKY19* silencing.

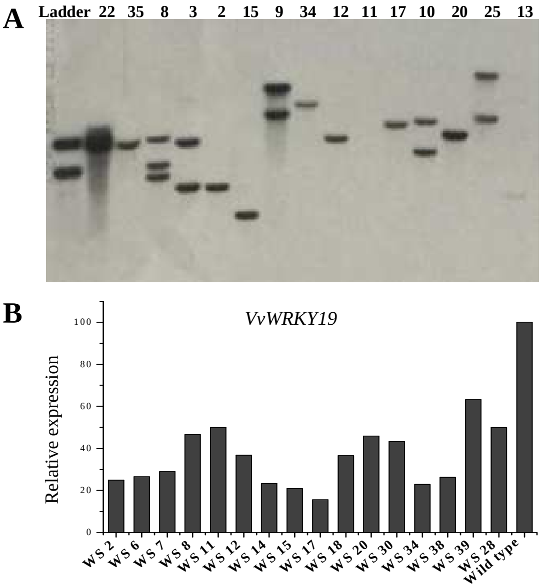

Figure 9. Selection of most silenced lines containing a single copy of the transgene.
A) Example of southern blot analysis to select the transgenic lines with a single copy of the transgene.
B) Real time RT-PCR analysis of *VvWRKY19* expression of leaves of silenced grapevines. The selected lines are WS 2, WS 11, WS 14, WS 17, WS 19 and WS 34.

For a deeper phenotypic characterization, six lines containing one copy of the transgene were selected (Figure 9). All transgenic lines presented dimension and growth similar to the wild type plant (Figure 10A). Significant anthocyanin accumulation was observed in epidermis of expanding leaves, which undergo necrosis during the growth (Figure 10C). This phenotypic effect was similar to the *VvMYB5a / VvMYB5b* silenced plants (Chapter 3), supporting the hypothesis that all these transcription factors can participate to the same regulatory cascade.

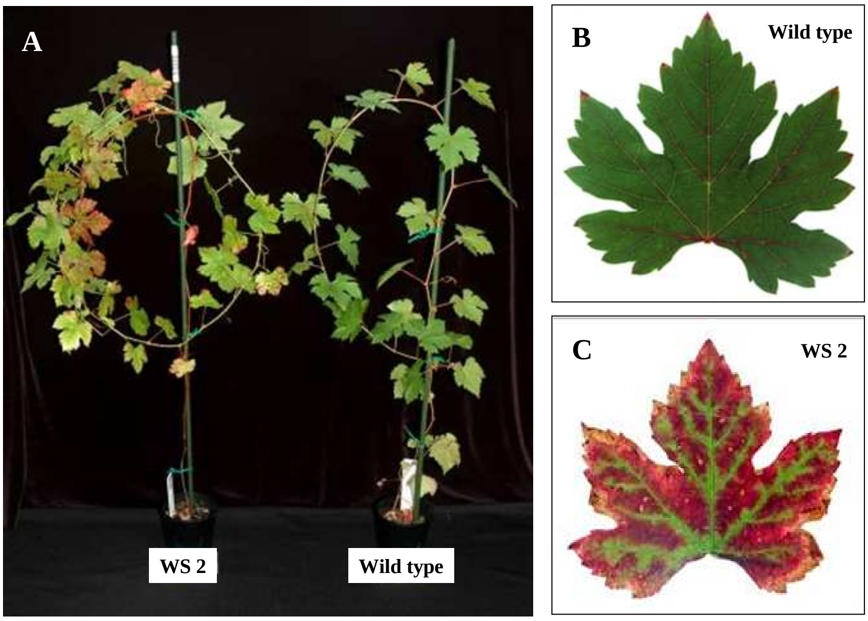

Figure 10. Phenotypes of grapevine silenced for *VvWRKY19* (line WS 2) in comparison to the wild type. Transgenic plants and the respective control were maintained in the same conditions in the glasshouse.
A) *VvWRKY19* silenced plants (line WS 2) presented growth and dimension similar to the wild type.
B-C) Leaves of wild type (**B**) and WS 2 transgenic plants (**C**) that show an increased pigmentation in epidermal cells.

Global transcriptome response analysis induced by *VvWRKY19* silencing

To dissect the pathways impaired by *VvWRKY19* silencing and to screen putative targets of VvWRKY19, a microarray analysis was performed on leaf RNA of *VvWRKY19* silencing and wild type plants, using the Nimblegen platform on three biological replicates. To identify the gene expression profiles with the greatest contribution to the differences between the transgenic and the wild type transcriptomes, a multiclass comparison analysis was carried out using Significance Analysis of Microarray (SAM) with a false discovery rate (FDR) of 0,1% (TMev 4.3). We identified 1314 genes modulated by *VvWRKY19* silencing with a fold change ≥ |2|, but to evaluate the principal modifications, we focused on genes with a fold change ≥ |5|, narrowing the analysis to 465. Among them, we identified a set of 334 down-regulated and 131 up-regulated genes (Supplementary table 1). All the transcripts were annotated against the V1 version of the 12X draft annotation of the grapevine genome (http://genomes.cribi.unipd.it/DATA/). To investigate the functional distribution of specifically modulated transcripts, we distributed them into 18 Gene Ontology (GO) functional categories and determined the percentage of genes in each category (Figure 11).

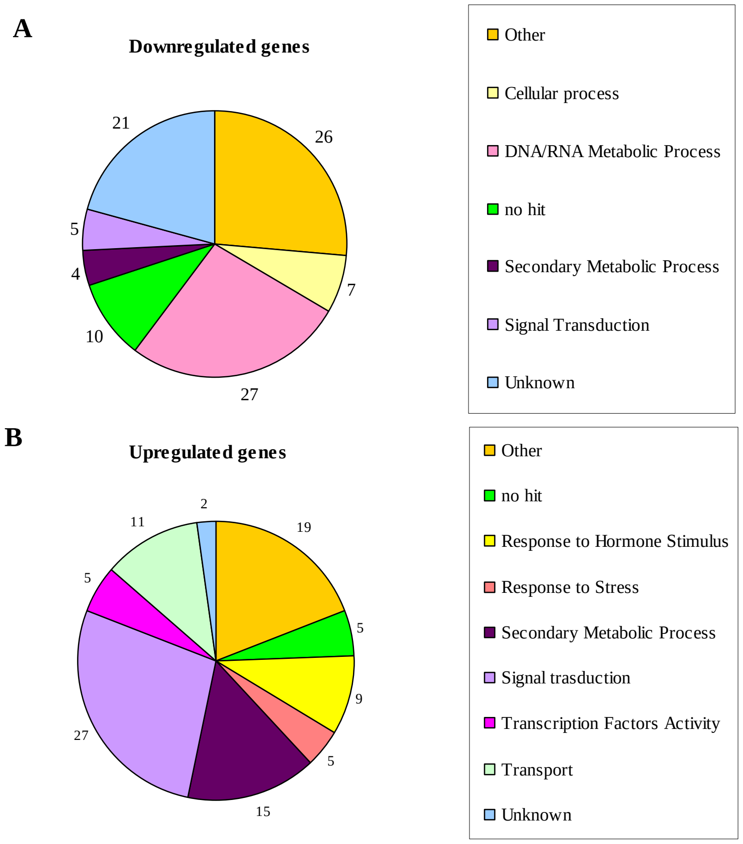

Figure 11. Distribution of down-regulated genes (**A**) and up-regulated genes (**B**) in *VvWRKY19* silenced plants into 18 Gene Ontology (GO) functional categories.

The most represented functional category shared among the down-regulated genes was “DNA/RNA Metabolic Process”, where several genes putatively involved in the cell cycle were grouped (Table 3). In fact, we identified gene for the replication of DNA, such as DNA polymerase α (VIT_07s0031g00470), cyclin B2 (VIT_03s0038g02800), cyclin-dependent kinase CDT1A (VIT_16s0039g00430) and DNA topoisomesase I (VIT_04s0008g02950) or for purines and pirimidines metabolism (Supplementary table 1). Genes representing mechanism of DNA repair were also grouped in this category, among which we identified a MAD2 factor of the mitotic spindle checkpoint system (VIT_01s0010g02430), a surveillance mechanism that prevents sister-chromatid separation and transition into anaphase during the mitosis. The “Cellular Process” category contained 23 genes assigned to the regulation of the cytoskeleton. Kinesin phragmoplast-associated protein 2 (VIT_05s0049g01350) and tubulin α (VIT_14s0108g00440), for example, were identified. In addition, the experiment revealed several genes linked to “Secondary metabolic processes”, mainly for terpenoid biosynthesis and to “Signal transduction”, including many calmodulin-binding proteins. In the “Transcription factor” category, the sequence VIT_15s0046g01130 was identified as the homolog of Arabidopsis *TRIPTYCHON*, a R3 MYB factor known to mediate lateral inhibition during trichome and root hair patterning (Schellmann *et al.*, 2002). We observed also genes having miscellaneous or unknown functions, and some of them do not even exhibit homology with other genes of the plant kingdom.

Regarding the genes induced by *VvWRKY19* silencing (Supplementary table 1), we identified 27 genes associated to the “Signal transduction” and 15 genes related to the “Secondary metabolic process”. Among the few flavonoid structural genes, flavonoid 3-monooxygenase (VIT_18s0001g11430) and anthocyanidin 3-O-glucosyltransferase (VIT_06s0004g06400) were represented. In the “Response to hormone stimulus” category, several genes showing homology with ethylene responsive factors were included as well as several transcripts involved in stress response.

Table 2. The 50 most repressed genes in *VvWRKY19* silenced leaves compared to the untransformed line.

SEQ_ID	GENE DESCRIPTION	FC
VIT_10s0003g03080	minichromosome maintenance protein 3	-24.3
VIT_18s0164g00050	no hit: unknown	-24.2
VIT_19s0027g01850	Unknown protein	-24.2
VIT_07s0005g03090	cyclin-dependent protein kinase CDT1A	-24.2
VIT_14s0083g00060	DDT domain-containing protein	-24.0
VIT_11s0118g00770	Unknown protein	-23.3
VIT_13s0064g01740	glycosyl hydrolase family 1 protein	-22.9
VIT_00s0313g00010	centromere protein	-22.9
VIT_13s0019g00800	histone H4	-22.8
VIT_09s0054g01700	C2-HC type zinc finger protein C.e-MyT1	-22.7
VIT_17s0000g04830	DNA Polymerase epsilon, subunit B	-22.6
VIT_00s0992g00030	calmodulin-binding	-22.6
VIT_18s0122g00550	cyclin-dependent kinase B2;1	-22.2
VIT_19s0090g01600	kinesin motor protein	-22.1
VIT_14s0128g00440	Tangled	-22.1
VIT_14s0068g00270	hydroxyproline-rich glycoprotein	-21.8
VIT_05s0020g01270	unknown	-21.4
VIT_08s0058g00540	Inflorescense meristem receptor-like kinase 2	-21.4
VIT_00s0184g00040	mini-chromosome maintenance protein MCM6	-21.3
VIT_17s0000g08460	carbonic anhydrase, chloroplast precursor	-21.3
VIT_08s0040g02590	kinesin motor protein	-21.2
VIT_09s0002g03350	no hit	-21.2
VIT_07s0031g00470	DNA polymerase alpha	-21.1
VIT_02s0025g04000	scarecrow transcription factor 29 (SCL29)	-21.0
VIT_18s0001g14320	Unknown protein	-21.0
VIT_01s0010g02430	mitotic spindle checkpoint protein (MAD2)	-21.0
VIT_12s0028g03190	reticulon family protein	-21.0
VIT_07s0005g01060	Histone H1	-20.9
VIT_04s0008g00530	Serine/threonine-protein kinase Aurora-1	-20.9
VIT_12s0028g03330	targeting protein	-20.8
VIT_02s0154g00600	pectinesterase family	-20.0
VIT_05s0020g02910	STE11 protein kinase homolog NPK1	-20.0
VIT_07s0129g01080	Microtubule end binding protein 1(EB1)	-19.7
VIT_00s2668g00010	F-box family protein	-19.7
VIT_02s0025g01350	primase polypeptide 1	-19.7
VIT_04s0008g05150	zinc finger (Ran-binding)	-19.4
VIT_08s0007g06500	Unknown protein	-19.3
VIT_18s0001g07550	kinesin family member 4/7/21/27	-19.2
VIT_13s0067g01420	cyclin 1b (CYC1b)	-19.1
VIT_13s0019g02710	Rho guanyl-nucleotide exchange factor ROPGEF5	-19.0
VIT_10s0092g00360	vacuolar protein sorting-associated protein	-18.9
VIT_05s0049g01720	no hit	-18.8
VIT_17s0000g09930	Unknown protein	-18.7
VIT_07s0255g00040	cell division cycle associated 7 CDCA7	-18.7
VIT_01s0026g00030	Unknown protein	-18.4
VIT_03s0038g02800	cyclin B2;4	-18.3
VIT_14s0068g02000	ribonucleotide reductase R2	-18.2
VIT_03s0088g00490	no hit	-18.2
VIT_00s0480g00070	polyphenol oxidase II, chloroplast precursor	-18.0

A comparison between the modulated genes observed in this experiment and the transcriptomic data of the microarray analysis performed using leaves silenced for *VvMYB5a / VvMYB5b* (Chapter 3) defined the genes that could belong to the same regulatory network. Supplementary table 2 presents the 71 genes that are modulated in the same way in *VvMYB5a / VvMYB5b* and *VvWRKY19* silenced plants. Among them, genes associated with DNA/RNA metabolic processees and transcription factor activity are the most represented. The homolog of *TRIPTYCHON* was found also in *VvMYB5a / VvMYB5b* silenced plants. All the other categories were less represented, while none of the common genes identified belonged to the secondary metabolism.

DISCUSSION

The WD40–bHLH–MYB transcription complex is responsible for the regulation of many epidermal cell fate processes in a range of plants. The high similarity between members of different species indicates that this regulatory complex is conserved and arose in the plant lineage. However, the variability of the controlled processes suggests a kind of flexibility of these regulators for the control of the target genes, resulting in the evolution of epidermal cell diversity in angiosperms. First insights into how this complex influences epidermal cell identity were provided by studies of Arabidopsis mutants (Walker *et al.*, 1999). The requirement of the regulatory complex was successively established also for other species, such as *Petunia hybrida* (de Vetten *et al.*, 1997; Quattrocchio *et al.*, 1999; Spelt *et al.*, 2000). Current works demonstrate that the regulatory complex is present also in grapevine (Hichri *et al.*, 2011), which is a perennial and woody plant evolutionary distinct from Arabidopsis or petunia.

In some species, in addition to the WD40–bHLH–MYB proteins, other transcription factors are part of the same regulative network. In Arabidopsis for example, AtTTG2 and AtGL2 have been identified as intermediate regulators of trichome outgrowth and branching, seed coat mucilage production and PA biosynthesis and root hair patterning, acting downstream of TTG1-MYB-bHLH complex (Rerie *et al.*, 1994; di Cristina *et al.*, 1996; Johnson *et al.*, 2002; Zhao *et al.*, 2008). Also in petunia another transcription factor PhPH3, a WRKY transcription factor homologue to AtTTG2, was identified to act downstream the complex driven by the MYB protein PhPH4 in the regulation of the vacuolar acidification of epidermal cells of petals. WRKY proteins are well known to be associated with the response to the pathogen infection (Eulgem *et al.*, 2000) and with the processes of mechanical wounding (Hara *et al.*, 2000) and senescence (Robatzek and Somssich, 2001). AtTTG2 and PhPH3 represent two of the few reports of WRKY factors involved in other developmental processes. Current studies revealed that AtTTG2 is able to complement the *ph3* mutation (Quattrocchio *et al.,* submitted), indicating that they are functional homologs.

By screening the last released genome prediction (Jaillon *et al.*, 2007), we were able to identified 52 WRKY genes, indicating that in grapevine this family appears as consistently decreased in size when compared to *Arabidopsis thaliana* (72 genes) or *Oryza sativa* (102 genes) (Riechmann *et al.*, 2000; Ross *et al.*, 2007). Among the 52 members, VvWRKY19 was identified as the closest homolog to AtTTG2 and PhPH3. It carries two tandemly repeated WRKY domains as all the members of group I (Eulgem *et al.*, 2000).

In order to study the regulative functions of VvWRKY19, we addressed the question if its role in grapevine could be related to those of AtTTG2 and PhPH3 in Arabidopsis and petunia, respectively. Even if *VvWRKY19* expression is ubiquitous in grapevine, it is mainly expressed in the earliest stages of development of many organs as leaves, buds, inflorescences and berries. Fully complementation of petunia *ph3* mutation demonstrated that VvWRKY19 is the real ortholog of PhPH3, given that it is able to restore completely the mutant phenotype, probably activating the expression of the structural genes for the vacuole acidification of the epidermal cells of petunia petals.

The first evidence that VvWRKY19 belongs to the regulative network of VvMYB5a and VvMYB5b was provided by the high correlation between *VvWRKY19* and *VvMYB5a* expression. We also showed that in *VvMYB5a / VvMYB5b* silencing grapevines, *VvWRKY19* expression appeared to be very weak, suggesting that VvWRKY19 acts downstream VvMYB5a and VvMYB5b in the regulatory cascade.

Another indication is provided by *VvMYB5a* overexpression in grape hairy root culture, which resulted in an induction of *VvWRKY19* expression. Combining all these data with the high similarity to AtTTG2 and PhPH3, in this work we assumed that VvWRKY19 could be an intermediate regulator acting downstream the complex and consequently this regulatory network might be conserved also in grapevine.

Yeast two-hybrid analyses demonstrated that as the ortholog PhPH3, VvWRKY19 interacts with the petunia AN11 and revealed that the same mechanism of action is conserved also in grapevine. VvWRKY19 in fact physically interacts with VvWD1. As previously demonstrated (unpublished data), PhPH3 is activated by PhAN11-PhAN1-PhPH4 complex so it works downstream in the regulatory network for the vacuolar acidification, but it also cooperates with the complex through a physical interaction with the regulatory protein WD40 AN11. The complementation of the petunia mutation *ph3* indicates that VvWRKY19 substitutes PhPH3, binding the regulatory complex driven by PhPH4. Based on our results, a model of interaction between the regulatory proteins of the complex in grapevine was proposed. We hyphotizesed that VvMYB5a and VvMYB5b factors bind directly the promoters of the structural genes with the R2R3 domain. The interaction with the bHLH factor (VvMYC1) could be mediated by the ID amino acidic sequence in the R3 repeat of the MYB domain and the N-terminal region of the bHLH factor, as was first demonstrated for the maize C1 and B regulators (Goff *et al.*, 1992; Deluc *et al.*, 2008; Hichri *et al.*, 2010). VvWD1, in turn, binds VvMYC1 and VvWRKY19 separately, indicating that it can be necessary to form a docking platform for the protein-protein interaction (Baudry *et al.*, 2004). The localization of VvWD1 in the nuclei shown by Matus (2010) confirmes its direct role in the transcription activation. However, the WD40 proteins are present predominantly in the cytoplasm, while the transcription factors are located inside the nucleus. Therefore, another explanation is that the VvWD1 facilitates the transport into the nucleus of the transcription factors and binds them only transiently, as previously proposed (Sompornpailin *et al.*, 2002).

Results of transient assays may partially confirm the model of regulation proposed and provide first indications about putative targets of the complex. In fact, the interaction of

VvWRKY19 with the regulatory complex may explain the weak enhancement of activation of Vv*ANR* and Vv*LAR1* promoters when regulated by VvMYB5a and VvMYB5b. The analysis of *VvANR* and *VvLAR1* promoter sequences revealed Wboxes, DNA sequences recognized by WRKY transcription factors. Thus, these preliminary functional data indicate that VvWRKY19 is involved in the regulation of PA biosynthesis. The expression profile of *VvWRKY19* correlates with *VvMYB5a* expression in the early steps of berry development in both seed and skin tissues, when the PA accumulate (Downey *et al.*, 2003). This data suggest that VvWRKY19 could play a part in the regulation of flavan-3-ols biosynthesis occurring during the early stages of berry development. Similar findings in regulating PA synthesis were described also for TTG2 of Arabidopsis (Johnson *et al.*, 2002).

As the homolog AtTTG2, VvWRKY19 could be also involved in the regulation of different processes. In order to clarify the specific roles of VvWRKY19, we analyzed the transcriptomic variations induced by silencing *VvWRKY19* in grape leaves by microarray. The analysis revealed the down-regulation of 334 genes and the induction of 131 genes. Looking for putative targets of VvWRKY19, first we focused our attention on the down-regulated genes. Among these, a small number of genes belonging to the secondary metabolism was identified and none of them could be clearly associated to the proanthocyanidin biosynthesis. Together with the weak enhancement of promoter activation observed in the transient assays, this result may indicate that VvWRKY19, possibly in cooperation with VvMYB5a and VvMYB5b, is required for the regulation of processes different from the flavonoid pathway in leaves. However, a role in PA synthesis during the berry development could not be excluded. Future phenotypic characterization of the fruits of the transgenic plants will provide more information about its eventual function in this specific pathway.

The most represented category of down-regulated genes was related to “DNA/RNA metabolism”. The majority of these genes could be associated to the cell cycle (phase G1 to M). For example, the subunit 7 of the ORC (Origin Recognition Complex), which binds in ATP-dependent manner to origins of replication, was found to be down-regulated with a FC = -13,4. ORC is involved in the recruitment of CDC (Cell Division

Cycle), CDT (DNA replication factor) and the MCM (Minichromosome Maintenance) proteins to form the Pre-replication complex during the G1 phase of the cell cycle. All these proteins were also identified. The cyclin D3 that regulates transition from G1 to S phase (Morgan, 2007) was also observed with a FC = - 9,2. DNA polymerases and DNA elicases usually active during the phase S of the cell cycle and related to the DNA replication, were noted. We found also genes encoding for histones, which are the only proteins synthesized during the this phase (Wu and Bonner, 1981). The following G2 checkpoint phase insures that cells don't initiate mitosis before they repair damaged DNA after replication. Linked to DNA repair mechanism, a gene for a RAD51C protein, which was previously shown in yeast to become associated with chromatin in response to DNA damage, was identified. Many kinesins were identified as motor proteins that move along microtubule filaments in the formation of the spindle apparatus during the mitosis. All these findings suggest a role of VvWRKY19 in the regulation of the cell cycle in leaves. Future experiments of promoter activation will provide information about the direct targets of VvWRKY19. Among the down-regulated genes, we focused our attention on the homolog of TRIPTYCHON (AtTRY), a negative regulator that competes with the MYB transcription factors AtGL1 and AtWER in the formation of the AtTTG1-MYB-bHLH complex during trichome and root hair patterning in Arabidopsis (Schellmann *et al.*, 2002). It is possible that in grape leaves the TRY homolog functions with the same mechanism, inhibiting the formation of the regulatory complex. The down-regulation of *TRY* could cause the assembly of the complex and therefore the synthesis of anthocyanins accumulated in leaves of the transgenic plants. On the other hand, this phenotypic effect may be due to stress condition caused by the silencing: this hypothesis is supported by the increased number of up-regulated genes related to “Stress conditions”. However, the absence of up-regulated genes for anthocyanin biosynthesis cannot explain the phenotype of the transgenic plants. As already hypothesized for *VvMYB5a / VvMYB5b* silencing plants (Chapter 3), it is possible that the leaf samples for the microarray analyses were collected when the phenotypic effects of the silencing were not visible and the expression of genes directly responsible for the phenotype observed could not be detected yet.

A comparison between the microarray results of plants silenced for *VvWRKY19* and *VvMYB5a / VvMYB5b* revealed a similar modulation of the gene expression. In fact, we identified many transcripts in both analyses, suggesting that VvWRKY19, VvMYB5a and VvMYB5b may belong to the same network and regulate the same pathways. Some of these genes are involved in the cell cycle. MAD2 (mitotic arrest deficient 2), an essential spindle checkpoint protein in the progression through the metaphase-to-anaphase transition (Li *et al.*, 1997) was found to be down-regulated with a FC of -21 and -2,8 in plants silenced for *VvWRKY19* and *VvMYB5a / VvMYB5b* expression, respectively. In chapter 3, we showed that the silencing of the MYB factors causes cell re-organization of the leaf tissues, reducing for example the epidermal cell layer in the veins. It is therefore presumable that the cell division is one process regulated by VvMYB5a, VvMYB5b and VvWRKY19. Further phenotypic characterization of *VvWRKY19* silencing plants will provide information about the role in the control of cell cycle.

Taken together, all these findings indicate that the regulatory network is conserved also in grapevine and that numerous processes seems to be regulated, flavonoid pathway in berry as well as cell division in vegetative tissues.

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SUPPLEMENTARY DATA

Supplementary table 1. Differentially expressed genes (> |5| fold) in *VvWRKY19* silenced leaves compared to the untransformed line.

SEQ_ID	GENE DESCRIPTION	FC
VIT_10s0003g03080	minichromosome maintenance protein 3	-24.3
VIT_18s0164g00050	no hit: unknown	-24.2
VIT_19s0027g01850	Unknown protein	-24.2
VIT_07s0005g03090	cyclin-dependent protein kinase CDT1A	-24.2
VIT_14s0083g00060	DDT domain-containing protein	-24.0
VIT_11s0118g00770	Unknown protein	-23.3
VIT_13s0064g01740	glycosyl hydrolase family 1 protein	-22.9
VIT_00s0313g00010	centromere protein	-22.9
VIT_13s0019g00800	histone H4	-22.8
VIT_09s0054g01700	C2-HC type zinc finger protein C.e-MyT1	-22.7
VIT_17s0000g04830	DNA Polymerase epsilon, subunit B	-22.6
VIT_00s0992g00030	calmodulin-binding	-22.6
VIT_18s0122g00550	cyclin-dependent kinase B2;1	-22.2
VIT_19s0090g01600	kinesin motor protein	-22.1
VIT_14s0128g00440	TANGLED	-22.1
VIT_14s0068g00270	hydroxyproline-rich glycoprotein	-21.8
VIT_05s0020g01270	unknown	-21.4
VIT_08s0058g00540	IMK2 (INFLORESCENCE MERISTEM RECEPTOR-LIKE KINASE 2)	-21.4
VIT_00s0184g00040	mini-chromosome maintenance protein MCM6	-21.3
VIT_17s0000g08460	carbonic anhydrase, chloroplast precursor	-21.3
VIT_08s0040g02590	kinesin motor protein	-21.2
VIT_09s0002g03350	no hit: MDN1, AAA ATPase containing von Willebrand factor type A (vWA) domain	-21.2
VIT_07s0031g00470	DNA polymerase alpha	-21.1
VIT_02s0025g04000	scarecrow transcription factor 29 (SCL29)	-21.0
VIT_18s0001g14320	Unknown protein	-21.0
VIT_01s0010g02430	mitotic spindle checkpoint protein (MAD2)	-21.0
VIT_12s0028g03190	reticulon family protein	-21.0
VIT_07s0005g01060	Histone H1	-20.9
VIT_04s0008g00530	Serine/threonine-protein kinase Aurora-1	-20.9
VIT_12s0028g03330	targeting protein	-20.8
VIT_02s0154g00600	pectinesterase family	-20.0
VIT_05s0020g02910	STE11 protein kinase homolog NPK1	-20.0
VIT_07s0129g01080	MICROTUBULE END BINDING PROTEIN 1 (EB1)	-19.7
VIT_00s2668g00010	F-box family protein	-19.7
VIT_02s0025g01350	primase polypeptide 1	-19.7
VIT_04s0008g05150	zinc finger (Ran-binding)	-19.4
VIT_08s0007g06500	Unknown protein	-19.3
VIT_18s0001g07550	kinesin family member 4/7/21/27	-19.2
VIT_13s0067g01420	cyclin 1b (CYC1b)	-19.1
VIT_13s0019g02710	Rho guanyl-nucleotide exchange factor ROPGEF5	-19.0
VIT_10s0092g00360	VPS2.2 SNF7: vacuolar protein sorting-associated protein (vesicle-mediated transport)	-18.9
VIT_05s0049g01720	no hit	-18.8
VIT_17s0000g09930	Unknown protein	-18.7
VIT_07s0255g00040	cell division cycle associated 7 CDCA7	-18.7
VIT_01s0026g00030	Unknown protein	-18.4
VIT_03s0038g02800	cyclin B2;4	-18.3
VIT_14s0068g02000	ribonucleotide reductase R2	-18.2
VIT_03s0088g00490	no hit	-18.2
VIT_00s0480g00070	polyphenol oxidase II, chloroplast precursor	-18.0
VIT_06s0004g01710	histone H3	-17.9
VIT_08s0007g05500	condensin subunit 1	-17.9
VIT_18s0001g10710	transcriptional factor B3	-17.8
VIT_14s0060g01480	unknown	-17.8
VIT_17s0000g03940	ferulate 5-hydroxylase	-17.8
VIT_14s0108g01630	Unknown protein	-17.7
VIT_08s0056g01260	Unknown protein	-17.6
VIT_17s0000g06900	DNA helicase SNF2 domain-containing protein	-17.5
VIT_18s0086g00410	auxin-binding protein ABP19	-17.4
VIT_06s0009g02610	Unknown protein	-17.3
VIT_19s0090g00500	Unknown protein	-17.3
VIT_01s0011g02800	RIC7 (ROP-INTERACTIVE CRIB MOTIF-CONTAINING PROTEIN 7)	-17.2
VIT_01s0011g03040	Unknown protein	-17.2
VIT_05s0062g00260	unknown	-17.1
VIT_12s0057g01510	Unknown protein	-17.1
VIT_05s0049g01390	Unknown protein	-17.1
VIT_04s0008g02570	REPLICON PROTEIN A (ATRPA2/ROR1/RPA2)	-17.1
VIT_12s0059g01070	chromosome associate protein subunit H	-17.0
VIT_11s0016g03640	Rac-like GTP-binding protein ARAC7 (GTPase protein ROP9)	-17.0
VIT_13s0064g00530	disease resistance protein (NBS-LRR class) RGH1	-16.8
VIT_04s0023g03360	Unknown protein	-16.7
VIT_17s0000g07630	DEL1 (DP-E2F-like 1)	-16.6
VIT_14s0108g00650	chromosome condensation protein	-16.3
VIT_15s0048g01730	aurora kinase 3	-16.3
VIT_11s0037g00910	no hit	-16.3
VIT_00s0160g00180	histone H3	-15.7
VIT_16s0022g02030	ERL1 (ERECTA 1)	-15.6
VIT_16s0022g01820	microtubule associated protein (MAP65/ASE1) PLEIADE	-15.5
VIT_18s0001g08700	kinesin ZCF125	-15.2
VIT_16s0050g00670	kinesin motor	-15.0
VIT_06s0004g01690	histone H3	-14.9
VIT_19s0027g01880	amino acid transport protein	-14.9
VIT_01s0010g01670	Zinc knuckle	-14.9
VIT_08s0007g06800	Chromomethylase	-14.7
VIT_07s0005g03990	Unknown protein	-14.6
VIT_11s0149g00190	transducin family protein / WD-40 repeat	-14.5

VIT_15s0046g00520	wax synthase	-14.4
VIT_01s0137g00750	protein phosphatase 2C	-14.2
VIT_10s0116g01610	monocopper oxidase SKS4 (SKU5 Similar 4)	-14.2
VIT_13s0067g03250	CENP-E like kinetochore protein	-14.1
VIT_03s0088g00050	serine carboxypeptitase 1	-14.0
VIT_00s0480g00100	catechol oxidase (EC 1.10.3.1) precursor - grape	-14.0
VIT_19s0015g01710	kinesin family member 22	-13.9
VIT_02s0025g04960	Unknown protein	-13.9
VIT_05s0029g00400	Kinesin PAKRP1L	-13.8
VIT_02s0025g01220	high mobility group HMG1/2	-13.7
VIT_06s0004g05920	Proliferating cell nuclear antigen (PCNA)	-13.6
VIT_17s0000g05830	Unknown protein	-13.5
VIT_11s0016g05800	no hit	-13.5
VIT_15s0046g01110	histone H3.2	-13.5
VIT_14s0006g00520	glucan endo-1,3-beta-glucosidase 3 precursor	-13.4
VIT_01s0026g02290	Origin recognition complex subunit 6	-13.4
VIT_01s0113g00500	argonaute	-13.3
VIT_18s0075g00270	no hit	-13.3
VIT_04s0044g00860	Protein BRUSHY 1 (Protein TONSOKU) (Protein MGOUN 3)	-13.3
VIT_12s0057g00500	Thymidine kinase	-13.3
VIT_15s0021g00210	EMB2411	-13.0
VIT_17s0000g04560	kinetochore protein	-13.0
VIT_18s0001g14170	cyclin-dependent protein kinase regulator CYCB2_4	-12.9
VIT_06s0004g01730	Unknown protein	-12.9
VIT_10s0116g00560	polyphenol oxidase II, chloroplast precursor	-12.9
VIT_00s0480g00080	polyphenol oxidase II, chloroplast precursor	-12.8
VIT_13s0147g00100	Nucleic acid-binding, OB-fold	-12.8
VIT_10s0003g01820	no hit	-12.8
VIT_00s0522g00010	linalool synthase	-12.6
VIT_18s0001g13590	leucine-rich repeat protein kinase	-12.5
VIT_18s0122g00470	spindle checkpoint protein Bub1b	-12.3
VIT_18s0001g09920	cyclin delta-3 (CYCD3_1)	-12.3
VIT_08s0007g05340	kinesin motor protein	-12.3
VIT_14s0219g00090	Unknown protein	-12.2
VIT_14s0128g00450	kinesin motor protein	-12.1
VIT_14s0006g03210	unknown	-12.1
VIT_10s0116g01600	monocopper oxidase SKS4 (SKU5 Similar 4)	-11.8
VIT_17s0000g07440	replication protein A 70 kDa DNA-binding subunit	-11.7
VIT_13s0067g03020	Unknown protein	-11.6
VIT_12s0034g01430	F-box family protein	-11.6
VIT_14s0006g01340	myb domain protein 113	-11.5
VIT_02s0087g00810	SWIB complex BAF60b domain-containing protein	-11.4
VIT_15s0048g00650	Unknown protein	-11.4
VIT_00s0266g00010	1,8-cineole synthase, chloroplast, putative OS=Ricinus communis	-11.4
VIT_00s0372g00070	linalool synthase	-11.4
VIT_14s0219g00030	zinc finger (C3HC4-type RING finger)	-11.3
VIT_05s0051g00930	DNA primase large subunit	-11.3
VIT_18s0072g00800	histone H3	-11.3
VIT_03s0091g00550	Unknown protein	-11.2
VIT_11s0016g02770	meiotic nuclear divisions 1	-11.2
VIT_10s0042g00100	superoxide dismutase, chloroplast	-11.2
VIT_16s0039g02400	protein kinase	-11.1
VIT_04s0008g02770	Syntaxin-related protein KNOLLE	-11.1
VIT_18s0001g06600	Unknown protein	-10.9
VIT_16s0050g02310	no hit	-10.7
VIT_03s0091g00650	no hit	-10.7
VIT_19s0014g00090	glucan endo-1,3-beta-glucosidase 4 precursor	-10.7
VIT_19s0015g00490	no hit	-10.6
VIT_08s0007g08030	no hit	-10.6
VIT_00s0572g00020	1,8-cineole synthase, chloroplast, putative OS=Ricinus communis	-10.6
VIT_06s0004g04370	histone H4	-10.5
VIT_05s0049g01350	Kinesin phragmoplast-associated kinesin-related protein 2 (PAKRP2)	-10.5
VIT_18s0001g14560	unknown	-10.4
VIT_00s0572g00010	linalool synthase	-10.3
VIT_07s0104g01270	kinase interacting family protein	-10.3
VIT_02s0234g00070	Unknown protein	-10.3
VIT_18s0072g00290	no hit	-10.2
VIT_00s0125g00200	no hit	-10.2
VIT_10s0003g05480	SEC14 cytosolic factor	-10.1
VIT_02s0025g01300	Unknown protein	-10.1
VIT_00s0372g00080	1,8-cineole synthase, chloroplast, putative OS=Ricinus communis	-10.0
VIT_00s1235g00010	Unknown protein	-10.0
VIT_00s0372g00040	1,8-cineole synthase, chloroplast, putative OS=Ricinus communis	-9.9
VIT_03s0088g00060	isoflavone reductase	-9.8
VIT_13s0019g00780	histone H4	-9.8
VIT_11s0016g03750	Myb-related protein 3R-1 (Plant c-MYB-like protein 1) MYB3R1	-9.8
VIT_16s0039g02430	no hit	-9.7
VIT_07s0031g00020	phosphoric monoester hydrolase	-9.7
VIT_06s0080g00760	Unknown protein	-9.7
VIT_09s0002g09020	no hit	-9.6
VIT_01s0011g02550	high mobility group HMG1/2	-9.4
VIT_06s0004g06300	cell division cycle 6	-9.4
VIT_14s0108g00670	chromosome condensation protein	-9.3
VIT_00s0199g00230	unknown	-9.3
VIT_08s0007g08020	no hit	-9.2
VIT_03s0180g00040	Cyclin D3_2	-9.2
VIT_07s0031g02770	Unknown protein	-9.0
VIT_07s0005g01030	Cellulose synthase CSLD5	-8.9
VIT_14s0030g01420	GCN5 N-acetyltransferase (GNAT)	-8.9
VIT_16s0039g00430	cyclin-dependent protein kinase CDT1A	-8.9
VIT_00s0227g00130	A/G-specific adenine glycosylase	-8.9
VIT_05s0077g01370	Unknown protein	-8.8
VIT_11s0016g03970	ubiquitin-conjugating enzyme E2 D/E	-8.8
VIT_00s1847g00010	Adenine phosphoribosyltransferase	-8.7
VIT_10s0003g05230	Tetratricopeptide repeat domain male sterility MS5	-8.7
VIT_13s0067g02030	IMK2 (INFLORESCENCE MERISTEM RECEPTOR-LIKE KINASE 2)	-8.7

VIT_08s0007g04950	xyloglucan endotransglucosylase/hydrolase 32	-8.7
VIT_15s0046g00590	wax synthase	-8.6
VIT_04s0008g06670	plastocyanin domain-containing protein	-8.6
VIT_15s0046g00660	wax synthase	-8.5
VIT_05s0020g03880	TSO1 (CHINESE FOR 'UGLY')	-8.5
VIT_14s0108g00440	Tubulin alpha chain	-8.5
VIT_18s0164g00010	Unknown protein	-8.5
VIT_05s0051g00490	outer membrane protein	-8.5
VIT_13s0064g00980	telomerase reverse transcriptase (TERT)	-8.4
VIT_13s0064g00740	lysine decarboxylase - like protein	-8.3
VIT_16s0013g00300	ATP-dependent DNA helicase	-8.3
VIT_18s0001g12030	uracil-DNA glycosylase	-8.2
VIT_07s0031g00770	Unknown protein	-8.2
VIT_07s0104g01420	glutaredoxin	-8.2
VIT_00s0516g00020	calcium-binding EF hand family	-8.1
VIT_03s0038g00150	beta-D-galactosidase	-8.0
VIT_13s0019g01210	Dirigent protein pDIR14	-8.0
VIT_08s0040g01790	laccase (diphenol oxidase)-like protein	-8.0
VIT_03s0091g00530	N-acetyltransferase ESCO2	-7.9
VIT_19s0027g00220	Unknown protein	-7.9
VIT_08s0058g01240	no hit	-7.9
VIT_08s0058g01250	Unknown protein	-7.7
VIT_16s0039g01780	DNA replication complex GINS protein PSF2	-7.7
VIT_00s0499g00040	FAS1 (FASCIATA 1)	-7.7
VIT_08s0007g07960	DnaJ homolog, subfamily C, member 9	-7.6
VIT_03s0038g01930	peptidyl-prolyl cis-trans isomerase ROC5 (ROTAMASE CYP 5)	-7.6
VIT_18s0001g02390	diphenol oxidase	-7.6
VIT_06s0009g03600	Wall-associated receptor kinase-like 14	-7.5
VIT_13s0019g05370	Histone H2B	-7.5
VIT_03s0038g04720	syntaxin 1B/2/3/4	-7.5
VIT_09s0002g04290	hydroxyphenylpyruvate reductase (HPPR)	-7.5
VIT_00s1616g00010	Unknown protein	-7.4
VIT_07s0129g00210	BT4 (BTB AND TAZ DOMAIN PROTEIN 4)	-7.4
VIT_14s0030g00250	Sugar transporter ERD6-like 3	-7.4
VIT_00s0847g00020	myrcene synthase	-7.4
VIT_04s0008g06590	kinetochore protein NUF2	-7.3
VIT_00s0302g00010	Unknown protein	-7.3
VIT_08s0007g05470	no hit	-7.2
VIT_04s0044g01680	chromatin remodeling 24	-7.2
VIT_14s0081g00200	AN3 (ANGUSITFOLIA3)	-7.1
VIT_13s0147g00230	Unknown protein	-7.1
VIT_17s0000g08910	Unknown protein	-7.0
VIT_01s0150g00540	Unknown protein	-7.0
VIT_00s0499g00020	Unknown protein	-7.0
VIT_19s0085g00490	FtsH protease 12	-7.0
VIT_00s0218g00050	kinesin motor protein	-7.0
VIT_17s0000g03750	peptidoglycan-binding LysM domain-containing protein	-7.0
VIT_03s0038g02370	mini-chromosome maintenance protein MCM10	-7.0
VIT_04s0008g04530	aminoacyl-tRNA synthetase, related	-6.9
VIT_13s0064g01440	SYN3 (Sister chromatid cohesion 1 protein 3)	-6.9
VIT_13s0019g00760	histone H4	-6.9
VIT_09s0002g02210	ADHESION OF CALYX EDGES (ACE)	-6.9
VIT_12s0057g00200	vesicle-associated membrane protein	-6.9
VIT_07s0005g01670	no hit	-6.9
VIT_16s0098g00480	lipase class 3	-6.9
VIT_01s0011g00080	universal stress protein (USP) family protein	-6.8
VIT_08s0040g02960	no hit	-6.8
VIT_14s0006g00050	Transposase, IS4	-6.8
VIT_02s0025g04830	copper chaperone for superoxide dismutase	-6.8
VIT_07s0031g01990	ribonucleotide reductase large subunit A	-6.8
VIT_08s0007g03190	Nucleic acid-binding, OB-fold	-6.8
VIT_13s0064g01020	Unknown protein	-6.7
VIT_17s0000g00250	Unknown protein	-6.6
VIT_05s0029g00640	Retrotransposon	-6.5
VIT_06s0004g03290	MaoC-like dehydratase	-6.5
VIT_05s0094g00520	Unknown protein	-6.5
VIT_00s0323g00050	invertase/pectin methylesterase inhibitor	-6.5
VIT_06s0004g02230	Unknown protein	-6.5
VIT_07s0005g00120	no hit	-6.4
VIT_09s0002g07090	Unknown protein	-6.4
VIT_01s0026g00670	Unknown protein	-6.4
VIT_15s0046g01130	myb TRIPTYCHON	-6.4
VIT_10s0116g01380	amino acid permease 2	-6.4
VIT_00s0245g00040	no hit	-6.3
VIT_00s0207g00180	Unknown protein	-6.3
VIT_04s0008g03780	Myb-related protein 3R-1 (Plant c-MYB-like protein 1) MYB3R1	-6.3
VIT_12s0028g00530	Cytochrome B561	-6.3
VIT_05s0051g00580	inosine triphosphate pyrophosphatase	-6.3
VIT_15s0046g00600	no hit	-6.2
VIT_14s0108g01320	plastocyanin domain-containing protein	-6.2
VIT_18s0001g12280	Unknown protein	-6.2
VIT_04s0023g01150	Unknown protein	-6.2
VIT_00s0207g00140	allergen V5/Tpx-1	-6.2
VIT_18s0001g05490	no hit	-6.2
VIT_11s0016g00590	invertase/pectin methylesterase inhibitor	-6.1
VIT_10s0003g00630	negative regulator of systemic acquired resistance (SNI1)	-6.1
VIT_14s0066g01590	NHL repeat-containing protein	-6.0
VIT_12s0028g01230	fanconi anemia, complementation group D2	-6.0
VIT_00s0199g00120	heat shock protein binding	-6.0
VIT_12s0028g01240	fanconi anemia, complementation group D2	-5.9
VIT_18s0001g14300	cytomatrix protein	-5.9
VIT_13s0019g03630	no hit	-5.9
VIT_00s0203g00160	Cyclin D-type	-5.9
VIT_00s0557g00010	integral membrane family protein	-5.9
VIT_07s0197g00010	Ribosomal protein L25	-5.9
VIT_01s0011g03490	3-ketoacyl-CoA synthase	-5.9

VIT_00s1247g00020	Ribosomal protein L25	-5.8
VIT_05s0020g01440	Unknown protein	-5.8
VIT_10s0116g01500	DNA helicase SNF2 domain-containing protein	-5.8
VIT_19s0014g02270	ribosomal protein L11 methyltransferase	-5.7
VIT_16s0098g01740	Unknown protein	-5.7
VIT_08s0007g03520	purple acid phosphatase 22- ATPAP22/PAP22	-5.7
VIT_18s0001g02500	Unknown protein	-5.7
VIT_09s0002g00800	zinc finger (CCCH-type) family protein	-5.7
VIT_02s0025g02030	no hit	-5.7
VIT_14s0068g02010	IMP dehydrogenase/GMP reductase	-5.6
VIT_03s0038g02180	glycosyl hydrolase family 10 protein	-5.6
VIT_06s0004g06730	microsomal omega-3 fatty acid desaturase	-5.6
VIT_07s0191g00250	exo-1,3-beta-glucanase	-5.6
VIT_00s0188g00140	amino acid permease 4	-5.6
VIT_01s0137g00720	lipase GDSL	-5.5
VIT_14s0083g01100	alpha-1,4-glucan-protein synthase 1	-5.5
VIT_01s0011g02840	cytochrome B561	-5.5
VIT_08s0007g06490	DNA polymerase delta, subunit B	-5.4
VIT_01s0011g04710	copper-binding family protein	-5.4
VIT_18s0001g03670	zinc finger (C2H2 type) family	-5.4
VIT_04s0008g02950	DNA topoisomerase I	-5.4
VIT_13s0073g00600	Receptor-like kinase 17	-5.4
VIT_18s0117g00030	no hit	-5.4
VIT_06s0080g00460	Nuclear transcription factor Y subunit B related	-5.4
VIT_15s0048g02610	ribosomal protein P1 acidic 60S	-5.4
VIT_12s0028g02630	Rac-like GTP-binding protein RAC2	-5.3
VIT_17s0000g02040	Unknown protein	-5.3
VIT_14s0066g02600	Unknown protein	-5.3
VIT_05s0077g02300	UDP-glucuronic acid decarboxylase 2	-5.3
VIT_08s0056g00820	cysteine synthase, chloroplast precursor	-5.3
VIT_13s0067g00360	no hit	-5.3
VIT_14s0030g01870	NIMA protein kinase	-5.3
VIT_04s0008g04710	beta-ketoacyl-CoA synthase	-5.2
VIT_03s0063g02240	PLATZ transcription factor	-5.2
VIT_13s0067g03650	histone H4	-5.2
VIT_16s0098g01070	calmodulin-binding protein family	-5.2
VIT_15s0048g00380	RAD51C DNA repair protein	-5.2
VIT_13s0019g04660	Amino acid permease	-5.2
VIT_08s0007g02620	Ribosomal protein S17 (RPS17A) 40S	-5.1
VIT_11s0016g03710	Abl interactor protein 1 (ABIL1)	-5.1
VIT_15s0021g01310	WRKY DNA-binding protein 12	-5.1
VIT_15s0021g01850	peptidyl-prolyl cis-trans isomerase FKBP-type FKB20-2	-5.1
VIT_08s0007g01840	no hit	-5.1
VIT_11s0016g02960	glucan endo-1,3-beta-glucosidase 4 precursor	-5.1
VIT_02s0025g02720	Unknown protein	-5.1
VIT_01s0026g00720	Unknown protein	-5.1
VIT_06s0004g01310	enoyl-[acyl-carrier-protein] reductase [NADH], chloroplast precursor	-5.0
VIT_13s0067g00840	TTN10 (TITAN 10)	-5.0
VIT_07s0005g02780	Unknown protein	-5.0
VIT_14s0060g02330	Actin binding FORMIN HOMOLOGY 1	-5.0
VIT_07s0031g02290	phosphate carrier protein	-5.0
VIT_15s0046g01360	no hit	-5.0
VIT_06s0004g07840	endo/excinuclease	-5.0
VIT_14s0030g00290	no hit	-5.0
VIT_10s0116g00590	pectinesterase family	-5.0
VIT_08s0007g05520	pentatricopeptide (PPR) repeat	-5.0
VIT_16s0115g00220	Myosin-like protein XIA	5.0
VIT_19s0015g00250	zinc transporter ZIP11	5.1
VIT_19s0014g04600	S-locus protein kinase	5.1
VIT_00s0540g00020	chitinase, class V	5.1
VIT_08s0007g07580	MYR1 (MYB-RELATED PROTEIN 1)	5.1
VIT_04s0008g00440	CLAVATA1 receptor kinase (CLV1)	5.1
VIT_15s0048g02070	BON2-associated protein (BAP2)	5.1
VIT_12s0035g00060	DNA (cytosine-5-)-methyltransferase	5.1
VIT_11s0016g05550	plastocyanin domain-containing protein	5.1
VIT_01s0137g00810	Acetylglucosaminyltransferase	5.1
VIT_12s0028g00670	Zinc knuckle	5.1
VIT_00s2380g00010	S-locus lectin protein kinase	5.1
VIT_16s0050g02710	no hit	5.2
VIT_11s0016g01660	phenylalanine ammonia-lyase	5.2
VIT_01s0011g02540	no hit	5.2
VIT_02s0025g04020	S-N-methylcoclaurine 3'-hydroxylase	5.2
VIT_07s0031g02390	phytoalexin-deficient 4 protein (PAD4)	5.3
VIT_12s0028g03010	glutaredoxin	5.4
VIT_16s0022g00080	HcrVf2 protein	5.5
VIT_18s0001g09730	S-domain receptor kinase	5.5
VIT_07s0129g00960	protein kinase	5.5
VIT_04s0008g00890	Bet v I allergen	5.6
VIT_06s0004g08190	Ethylene-responsive transcription factor CYTOKININ RESPONSE FACTOR 4	5.6
VIT_11s0149g00290	receptor kinase CHRK1	5.6
VIT_09s0018g00240	WRKY DNA-binding protein 40	5.6
VIT_09s0002g03060	leucine-rich repeat protein kinase	5.6
VIT_13s0047g00210	flavonol synthase	5.7
VIT_10s0003g01410	CBL-interacting protein kinase 20 (CIPK20)	5.7
VIT_06s0004g04470	heat shock cognate 70 kDa protein 1	6.0
VIT_16s0013g00890	ethylene-responsive element binding factor	6.0
VIT_08s0040g00820	CYP94A1	6.0
VIT_00s0662g00040	Ethylene-responsive transcription factor RELATED TO APETALA2 4	6.0
VIT_17s0000g09570	CYP71A26	6.0
VIT_11s0016g00870	strictosidine synthase (YLS2)	6.1
VIT_00s0218g00200	UDP-glucuronosyl/UDP-glucosyltransferase	6.1
VIT_08s0058g00240	myb family	6.1
VIT_05s0102g00200	Unknown protein	6.1
VIT_06s0004g06080	proton-dependent oligopeptide transport (POT) family protein	6.2
VIT_00s1553g00010	subtilisin-like proteinase AIR3	6.2
VIT_00s0662g00030	Ethylene-responsive transcription factor RELATED TO APETALA2 4	6.2

VIT_18s0001g09750	S-domain receptor kinase	6.3
VIT_03s0017g01140	cytokinin-O-glucosyltransferase 2	6.4
VIT_06s0004g05070	zinc transporter (ZIP2)	6.5
VIT_10s0116g01860	MATE efflux family protein	6.5
VIT_00s0772g00010	protein kinase	6.5
VIT_16s0148g00110	receptor kinase homolog LRK10	6.5
VIT_16s0148g00260	Ser/Thr receptor-like kinase1	6.6
VIT_07s0005g00820	AP2-like ethylene-responsive transcription factor	6.6
VIT_02s0025g04930	CDI3/OZS1/RCD3/SLAC1 (SLOW ANION CHANNEL-ASSOCIATED 1)	6.7
VIT_09s0054g01440	beta-amyrin synthase	6.8
VIT_19s0014g02190	tyrosine aminotransferase	6.8
VIT_07s0031g00530	MAPKKK21	6.8
VIT_00s0463g00040	no hit	6.8
VIT_03s0097g00460	Geraniol 10-hydroxylase	6.9
VIT_19s0014g04470	S-locus protein kinase	6.9
VIT_10s0003g01960	RKF1 (RECEPTOR-LIKE KINASE IN FLOWERS 1)	6.9
VIT_05s0094g01210	amine oxidase	7.0
VIT_05s0077g01700	no hit	7.0
VIT_12s0028g03520	S-receptor kinase	7.1
VIT_00s0366g00020	CRK10 (CYSTEINE-RICH RLK10)	7.3
VIT_02s0025g01790	cellulose synthase CSLG3	7.4
VIT_12s0034g02590	Leucine Rich Repeat receptor-like kinase	7.4
VIT_00s0218g00170	UDP-rhamnose/rhamnosyltransferase	7.4
VIT_04s0044g01610	CYP82C1p	7.5
VIT_19s0014g04620	S-locus receptor kinase	7.5
VIT_12s0057g00950	no hit	7.5
VIT_18s0089g00510	Isopentenyltransferase 1	7.5
VIT_18s0001g11470	CyP82A3	7.6
VIT_00s1566g00010	UDP-rhamnose/rhamnosyltransferase	7.7
VIT_18s0041g01290	Serine/threonine-protein kinase CCR4	7.7
VIT_01s0026g00880	transducin protein	7.8
VIT_05s0077g01300	Aldo-keto reductase	7.8
VIT_00s0218g00150	UDP-rhamnose/rhamnosyltransferase	7.8
VIT_09s0054g01520	beta-amyrin synthase	7.9
VIT_09s0002g03010	light repressible receptor protein kinase	8.0
VIT_05s0051g00820	nitrate transporter	8.0
VIT_16s0148g00070	receptor-like protein kinase	8.1
VIT_18s0001g11520	flavonoid 3-monooxygenase	8.1
VIT_16s0050g01400	C2 domain-containing protein	8.3
VIT_07s0031g02740	Auxin-responsive SAUR12	8.5
VIT_01s0137g00460	RGLG2 (RING DOMAIN LIGASE2)	8.5
VIT_18s0001g10330	subtilisin protease	8.5
VIT_18s0001g11490	CYP82C1p	8.5
VIT_09s0002g02990	light repressible receptor protein kinase	8.7
VIT_17s0119g00260	nitrate transporter 2	9.0
VIT_03s0017g01550	CRK10 (CYSTEINE-RICH RLK10)	9.2
VIT_02s0012g00170	1,4-alpha-D-glucan maltohydrolase	9.3
VIT_18s0001g09840	S-receptor kinase	9.3
VIT_18s0001g09800	S-receptor kinase	9.4
VIT_08s0040g02200	peroxidase ATP2a	9.5
VIT_17s0000g03330	receptor serine/threonine kinase PR5K	9.6
VIT_13s0074g00680	ABC transporter G member 22	9.7
VIT_07s0031g01710	WRKY DNA-binding protein 51	9.8
VIT_06s0004g06400	anthocyanidin 3-O-glucosyltransferase	9.8
VIT_00s1916g00010	DNA binding	10.1
VIT_19s0014g04530	S-locus receptor kinase	10.1
VIT_05s0029g00140	Ethylene-responsive transcription factor ERF062	10.2
VIT_08s0056g00800	myb domain protein 60	10.2
VIT_08s0058g00140	AMMONIUM TRANSPORTER 2	10.2
VIT_00s0374g00020	S-locus lectin protein kinase	10.3
VIT_18s0001g09770	S-locus receptor protein kinase	10.3
VIT_16s0098g00860	Flavanone 3-hydroxylase	10.5
VIT_18s0001g09740	S-locus receptor protein kinase	10.5
VIT_06s0004g06380	UDP-glucose: anthocyanidin 5,3-O-glucosyltransferase	10.5
VIT_09s0002g03020	light repressible receptor protein kinase	10.6
VIT_00s0218g00190	UDP-rhamnose:rhamnosyltransferase	10.7
VIT_16s0050g01610	UDP-glycosyltransferase 88A4	10.7
VIT_03s0063g00450	Alpha-amylase	11.1
VIT_03s0167g00190	CYP714A1	11.1
VIT_01s0011g06490	deoxymugineic acid synthase	11.3
VIT_18s0001g08840	serine carboxypeptidase S10	11.4
VIT_08s0007g02450	aspartyl protease	11.4
VIT_06s0080g01200	harpin-induced family protein / HIN1 family protein / harpin-responsive family protein	11.7
VIT_07s0005g03260	transcription factor TSRF1	11.9
VIT_08s0007g06310	myb family	12.1
VIT_17s0000g03390	Calmodulin binding protein	12.4
VIT_19s0014g04630	S-locus protein kinase	13.3
VIT_18s0166g00260	no hit	13.8
VIT_05s0077g00900	no hit	14.3
VIT_00s0625g00020	ABC transporter G member 7	15.0
VIT_09s0054g01360	cycloartenol synthase	15.3
VIT_04s0008g04810	unknown	15.7
VIT_00s0822g00010	WHITE-BROWN COMPLEX HOMOLOG PROTEIN 11	15.7
VIT_01s0011g05380	pectinesterase family	16.4
VIT_08s0058g00450	substrate carrier, Mitochondrial	16.7
VIT_06s0061g00360	UDP-glucoronosyl and UDP-glucosyl transferase	18.1
VIT_01s0127g00470	galactinol synthase	18.4
VIT_18s0001g11430	flavonoid 3-monooxygenase	20.2
VIT_08s0040g02180	Mlo3	22.2
VIT_01s0011g04700	heavy-metal-associated domain-containing protein / copper chaperone (CCH)	24.8
VIT_11s0052g01250	xyloglucan endotransglucosylase/hydrolase 23	27.0
VIT_02s0025g03310	arsenite transport protein (ArsB)	41.6

Supplementary table 2. Down-regulated genes in *VvWRKY19* and *VvMYB5a / VvMYB5b* silenced leaves compared to the untransformed line.

SEQ_ID	GENE DESCRIPTION	FC VvWRKY19	FC VvMYB5a/5b
VIT_18s0164g00050	no hit: unknown	-24.2	-6.8
VIT_14s0128g00440	TANGLED	-22.1	-1.9
VIT_14s0068g00270	hydroxyproline-rich glycoprotein	-21.8	-2.8
VIT_17s0000g08460	carbonic anhydrase. chloroplast precursor	-21.3	-9.3
VIT_18s0001g14320	Unknown protein	-21.0	-3.3
VIT_01s0010g02430	mitotic spindle checkpoint protein (MAD2)	-21.0	-2.8
VIT_07s0129g01080	MICROTUBULE END BINDING PROTEIN 1 (EB1)	-19.7	-3.7
VIT_04s0008g05150	zinc finger (Ran-binding)	-19.4	-3.2
VIT_08s0007g06500	Unknown protein	-19.3	-3.4
VIT_14s0108g01630	Unknown protein	-17.7	-2.2
VIT_18s0086g00410	auxin-binding protein ABP19	-17.4	-16.7
VIT_04s0008g02570	REPLICON PROTEIN A (ATRPA2/ROR1/RPA2)	-17.1	-3.3
VIT_11s0037g00910	no hit	-16.3	-3.6
VIT_16s0050g00670	kinesin motor	-15.0	-2.7
VIT_19s0027g01880	amino acid transport protein	-14.9	-8.3
VIT_07s0005g03990	Unknown protein	-14.6	-2.8
VIT_15s0046g00520	wax synthase	-14.4	-5.5
VIT_03s0088g00050	serine carboxypeptitase 1	-14.0	-6.8
VIT_02s0025g04960	Unknown protein	-13.9	-2.6
VIT_15s0046g01110	histone H3.2	-13.5	-3.6
VIT_18s0075g00270	no hit	-13.3	-17.0
VIT_17s0000g04560	kinetochore protein	-13.0	-3.4
VIT_13s0147g00100	Nucleic acid-binding. OB-fold	-12.8	-3.1
VIT_18s0122g00470	spindle checkpoint protein Bub1b	-12.3	-3.9
VIT_14s0006g03210	Unknown	-12.1	-19.6
VIT_14s0006g01340	myb domain protein 113	-11.5	-3.4
VIT_02s0087g00810	SWIB complex BAF60b domain-containing protein	-11.4	-2.9
VIT_14s0219g00030	zinc finger (C3HC4-type RING finger)	-11.3	-3.3
VIT_18s0001g06600	Unknown protein	-10.9	-4.2
VIT_16s0050g02310	no hit	-10.7	-2.4
VIT_03s0091g00650	no hit	-10.7	-4.3
VIT_19s0015g00490	no hit	-10.6	-1.4
VIT_08s0007g08030	no hit	-10.6	-2.8
VIT_18s0072g00290	no hit	-10.2	-2.1
VIT_00s1235g00010	Unknown protein	-10.0	-1.7
VIT_09s0002g09020	no hit	-9.6	-1.7
VIT_00s0199g00230	Unknown	-9.3	-3.7
VIT_08s0007g08020	no hit	-9.2	-2.1
VIT_07s0031g02770	Unknown protein	-9.0	-2.7
VIT_00s1847g00010	Adenine phosphoribosyltransferase	-8.7	-1.8
VIT_08s0007g04950	xyloglucan endotransglucosylase/hydrolase 32	-8.7	-3.3
VIT_07s0031g00770	Unknown protein	-8.2	-5.8
VIT_19s0027g00220	Unknown protein	-7.9	-1.4
VIT_08s0007g07960	DnaJ homolog. subfamily C. member 9	-7.6	-2.9
VIT_06s0009g03600	Wall-associated receptor kinase-like 14	-7.5	-3.3
VIT_03s0038g04720	syntaxin 1B/2/3/4	-7.5	-3.9
VIT_00s1616g00010	Unknown protein	-7.4	-3.7
VIT_14s0081g00200	AN3 (ANGUSITFOLIA3)	-7.1	-3.5
VIT_00s0218g00050	kinesin motor protein	-7.0	-2.2
VIT_08s0007g03190	Nucleic acid-binding. OB-fold	-6.8	-3.0
VIT_17s0000g00250	Unknown protein	-6.6	-3.1
VIT_06s0004g03290	MaoC-like dehydratase	-6.5	-3.2
VIT_07s0005g00120	no hit	-6.4	-2.7
VIT_09s0002g07090	Unknown protein	-6.4	-2.2
VIT_15s0046g01130	myb TRIPTYCHON	-6.4	-3.2
VIT_12s0028g00530	Cytochrome B561	-6.3	-2.9
VIT_18s0001g05490	no hit	-6.2	-2.1
VIT_18s0001g14300	cytomatrix protein	-5.9	-3.8
VIT_05s0020g01440	Unknown protein	-5.8	-2.5
VIT_16s0098g01740	Unknown protein	-5.7	-2.1
VIT_08s0007g03520	purple acid phosphatase 22- ATPAP22/PAP22	-5.7	-3.2
VIT_09s0002g00800	zinc finger (CCCH-type) family protein	-5.7	-3.7
VIT_06s0004g06730	microsomal omega-3 fatty acid desaturase	-5.6	-2.3
VIT_01s0011g04710	copper-binding family protein	-5.4	-2.0
VIT_18s0117g00030	no hit	-5.4	-2.9
VIT_06s0080g00460	Nuclear transcription factor Y subunit B related	-5.4	-2.6
VIT_12s0028g02630	Rac-like GTP-binding protein RAC2	-5.3	-1.8
VIT_13s0067g00360	no hit	-5.3	-2.1
VIT_08s0007g01840	no hit	-5.1	-3.1
VIT_13s0067g00840	TTN10 (TITAN 10)	-5.0	-4.7
VIT_07s0005g02780	Unknown protein	-5.0	-2.0
VIT_06s0004g07840	endo/excinuclease	-5.0	-2.0