The Basic Leucine Zipper Transcription Factor ABSCISIC ACID RESPONSE ELEMENT-BINDING FACTOR2 Is an Important Transcriptional Regulator of Abscisic Acid-Dependent Grape Berry Ripening Processes[1][W][OPEN]

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In grape (*Vitis vinifera*), abscisic acid (ABA) accumulates during fruit ripening and is thought to play a pivotal role in this process, but the molecular basis of this control is poorly understood. This work characterizes ABSCISIC ACID RESPONSE ELEMENT-BINDING FACTOR2 (VvABF2), a grape basic leucine zipper transcription factor belonging to a phylogenetic subgroup previously shown to be involved in ABA and abiotic stress signaling in other plant species. *VvABF2* transcripts mainly accumulated in the berry, from the onset of ripening to the harvesting stage, and were up-regulated by ABA. Microarray analysis of transgenic grape cells overexpressing *VvABF2* showed that this transcription factor up-regulates and/or modifies existing networks related to ABA responses. In addition, grape cells overexpressing *VvABF2* exhibited enhanced responses to ABA treatment compared with control cells. Among the VvABF2-mediated responses highlighted in this study, the synthesis of phenolic compounds and cell wall softening were the most strongly affected. VvABF2 overexpression strongly increased the accumulation of stilbenes that play a role in plant defense and human health (resveratrol and piceid). In addition, the firmness of fruits from tomato (*Solanum lycopersicum*) plants overexpressing *VvABF2* was strongly reduced. These data indicate that VvABF2 is an important transcriptional regulator of ABA-dependent grape berry ripening.

Grape (*Vitis vinifera*) is a nonclimacteric fruit that is important worldwide for wine production and fresh consumption. The ripening of grape berry is a complex process involving the catabolism of organic acids, the accumulation of soluble sugars, flavonoids, and aromatic compounds, and an increase in berry softness (Seymour et al., 1993; Ribéreau-Gayon et al., 2000). Berry composition and quality largely depend on the processes that coordinate these biochemical, physiological, and anatomical changes during ripening. While major progress has been made in the understanding of the key mechanisms supporting ethylene-mediated ripening of climacteric fruits (e.g. tomato [*Solanum lycopersicum*]; Adams-Phillips et al., 2004; Giovannoni, 2004, 2007), the events controlling the ripening of nonclimacteric fruits are less investigated and known.

Grape berry ripening involves the integration of multiple hormone signals. Classical plant hormones such as abscisic acid (ABA), auxin (indole-3-acetic acid [IAA]), brassinosteroids, and, to a lesser extent, ethylene have previously been implicated in this process (Davies et al., 1997; Chervin et al., 2004, 2008; Symons et al., 2006). ABA content of grape berries gradually and strongly increases just before the onset of ripening, called véraison (Coombe and Hale, 1973; Scienza et al., 1978; Davies et al., 1997; Antolín et al., 2003; Deluc et al., 2009). In relation with its accumulation profile, numerous reports suggested that ABA may play a major role in controlling several ripening-associated processes of grape berry, including coloration, sugar accumulation, and softening (Coombe, 1992; Davies et al., 1997; Giovannoni, 2001; Rodrigo et al., 2003; Yu

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et al., 2006; Wheeler et al., 2009; Gambetta et al., 2010; Giribaldi et al., 2010; Gagné et al., 2011).

In recent years, much progress has been made in the understanding of ABA signal transduction pathways in Arabidopsis (*Arabidopsis thaliana*; Umezawa et al., 2009; Cutler et al., 2010; Weiner et al., 2010). The ABA signal is perceived by multiple receptors (Shen et al., 2006; Liu et al., 2007; Fujii et al., 2009; Melcher et al., 2009; Miyazono et al., 2009; Nishimura et al., 2009; Pandey et al., 2009; Santiago et al., 2009; Shang et al., 2010; Chai et al., 2011; Jia et al., 2011; Sun et al., 2011), which trigger downstream signaling cascades resulting in physiological effects. Among these ABA receptors, only the PYRABACTIN RESISTANCE1 (PYR1)/PYR1-LIKE (PYL)/REGULATORY COMPONENTS OF ABA RECEPTORS (RCAR) proteins have been well characterized. ABA promotes the interaction of its receptor (PYR1) to PROTEIN PHOSPHATASE2C (PP2C), which results in the inactivation of PP2C and the activation of SUCROSE NONFERMENTING-RELATED KINASE2 (SnRK2). Activated SnRK2 turns on ABA signaling through the phosphorylation of downstream targets such as AREB/ABF (for ABA-response element-binding factor) transcription factors, which in turn activate several sets of genes (Fujii et al., 2009).

AREB/ABFs are ABA-responsive transcription factors containing a basic leucine zipper family (bZIP)-type DNA-binding domain that binds the ABA-responsive element (T/CACGTGGC) and have a pivotal role in ABA-dependent gene activation (Choi et al., 2000; Uno et al., 2000; Hattori et al., 2002; Kang et al., 2002; Gómez-Porras et al., 2007). Among the AREB/ABFs, AREB1/ABF2, AREB2/ABF4, and ABF3 were shown to be master transcription factors that cooperatively regulate ABA response element (ABRE)-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation (Yoshida et al., 2010).

Although the ABA regulation of fleshy fruit development has been studied extensively, the molecular mechanisms underlying ABA perception and signal transduction in these fruits remain unclear. In this study, we characterized and functionally studied VvABF2, an AREB/ABF-like transcription factor from grape, previously described as GRAPE RIPENING-INDUCED PROTEIN55 (GRIP55; Davies and Robinson, 2000), which accumulates in cv Cabernet Sauvignon berries from véraison until the end of the ripening phase. *VvABF2* expression is induced by ABA. A transcriptomic analysis made on transgenic grape cells overexpressing *VvABF2* treated or not with ABA led to the identification of putative target genes for VvABF2 mediated by ABA-dependent or -independent pathways. *VvABF2* overexpression in grape cells also strongly increased the accumulation of stilbenes. Its overexpression in tomato accelerated fruit ripening. Altogether, this work shows that VvABF2 is involved in the ABA signaling pathway and may affect grape berry ripening by activating several processes, including the synthesis of some phenolic compounds and fruit softening.

RESULTS

Identification of VvABF2, a bZIP Transcription Factor from Grape

Data from the literature describing changes in mRNA profiles during grape berry ripening led to the identification of a number of GRIP complementary DNAs (cDNAs) whose transcripts accumulate during berry development (Davies and Robinson, 2000). Among these GRIP genes, GRIP55 (VIT_18s0001g10450, Q9M4H1), a transcription factor of the bZIP family, was selected for further analysis. This choice was driven by the fact that members from this family are responsive to ABA (Choi et al., 2000; Uno et al., 2000; Amir Hossain et al., 2010), a hormone playing a crucial role in grape berry development and ripening (Giribaldi et al., 2010; Koyama et al., 2010). The *GRIP55* full-length cDNA was amplified by PCR using mRNAs extracted from cv Cabernet Sauvignon grape berries at véraison. The corresponding transcript is 1,341 bp long and encodes a protein of 447 amino acids. Amino acid sequence analysis further confirmed that this protein belongs to the transcription factors of the bZIP family characterized by a typical DNA basic-binding region, a Leu zipper dimerization motif located at the C-terminal region (amino acids 367–418; Jakoby et al., 2002), and conserved domains predicted as phosphorylation sites involved in stress or ABA signaling (Furihata et al., 2006; Fig. 1). A phylogenetic analysis revealed that this protein belongs to group A of bZIP transcription factors, previously shown to be involved in ABA and abiotic stress signaling (Choi et al., 2000; Uno et al., 2000; Amir Hossain et al., 2010; Fig. 2). Compared with Arabidopsis, bZIP group A from grape contains six members, among which only two belong to the possible groups of orthologs A5 (Corrêa et al., 2008; Fig. 1). The close homology of GRIP55 with AtAREB1/AtABF2 from Arabidopsis led us to rename this protein as VvABF2 (Figs. 1 and 2).

Expression Analysis of *VvABF2* in Grapevine and in Response to ABA

The expression profile of *VvABF2* was determined in different grapevine organs by real-time reverse transcription (RT)-PCR with RNA extracted from cv Cabernet Sauvignon roots, stems, leaves, flowers, and mature berries (11 weeks after flowering [WAF]). *VvABF2* was ubiquitously expressed in the different grape organs, but its relative expression depended on the organ (Fig. 3A). In decreasing order, *VvABF2* transcript accumulation was highest in ripening berries, stems, leaves, roots, and inflorescences.

VvABF2 transcript accumulation was also assessed during berry development (Fig. 3B). Interestingly, *VvABF2* expression increased just before the onset of grape berry ripening (8 WAF). Additionally, *VvABF2* transcripts accumulated more than 2-fold during the ripening stage (9–15 WAF) when compared with the herbaceous phase (2–7 WAF; Fig. 3B).

VvABF2 gene expression was also analyzed in the different berry compartments (seeds, pulp, and skin)

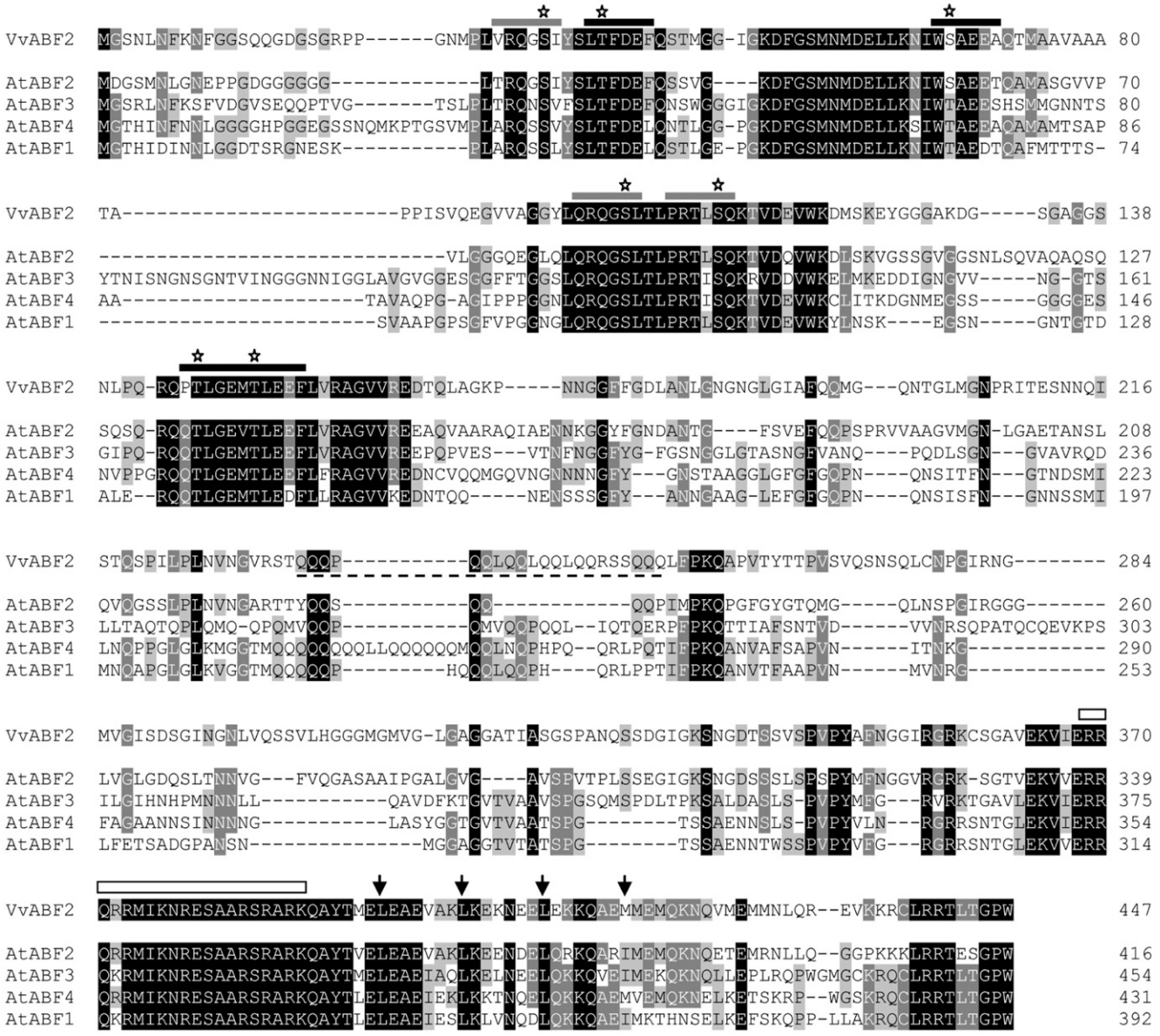

Figure 1. Sequence analysis of VvABF2. Full-length sequence comparison of VvABF2 and its closest orthologs from Arabidopsis, AtABF2 (AF093445), AtABF3 (AF093546), AtABF4 (AF093547), and AtABF1 (AF093544), using the Clustal Omega program (Sievers et al., 2011). Conserved residues are shaded in black, conserved residues in four out of five of the sequences are indicated in dark gray shading, and conserved residues in three out of five of the sequences are shown by a light gray shading. The basic regions and the Leu repeats are indicated by white rectangles and arrows, respectively. The Gln-rich region, commonly found in transcriptional activation domains, is underlined (dashed line) for VvABF2. The recognition sites for calmodulin-dependent protein kinase II (XRXXSX) and casein kinase II [X(S/T)XX(D/E)X] are indicated, respectively, by gray and black lines on the top of the alignment. Putative phosphorylated amino acids in the VvABF2 sequence are marked by stars.

after véraison. *VvABF2* transcripts were more abundant in both seeds and skin than in pulp (Fig. 3C).

The phylogenetic analysis revealed that this protein belongs to group A of bZIP transcription factors, previously shown to be involved in ABA signaling (Amir Hossain et al., 2010). Therefore, we investigated the effect of ABA on *VvABF2* expression by treating Cabernet Sauvignon berry (CSB) cell suspensions with 20 μM ABA. In these treated cells, *VvABF2* transcripts accumulated within the first 2 h of treatment and reached a maximum at 6 h, before slightly declining until 24 h after ABA supply (Fig. 3D).

VvABF2 Encodes a Functional Transcription Factor

The presence of a bipartite nuclear localization signal between amino acids 357 and 373 suggests that VvABF2 is probably targeted to the nucleus (Fig. 1). To confirm

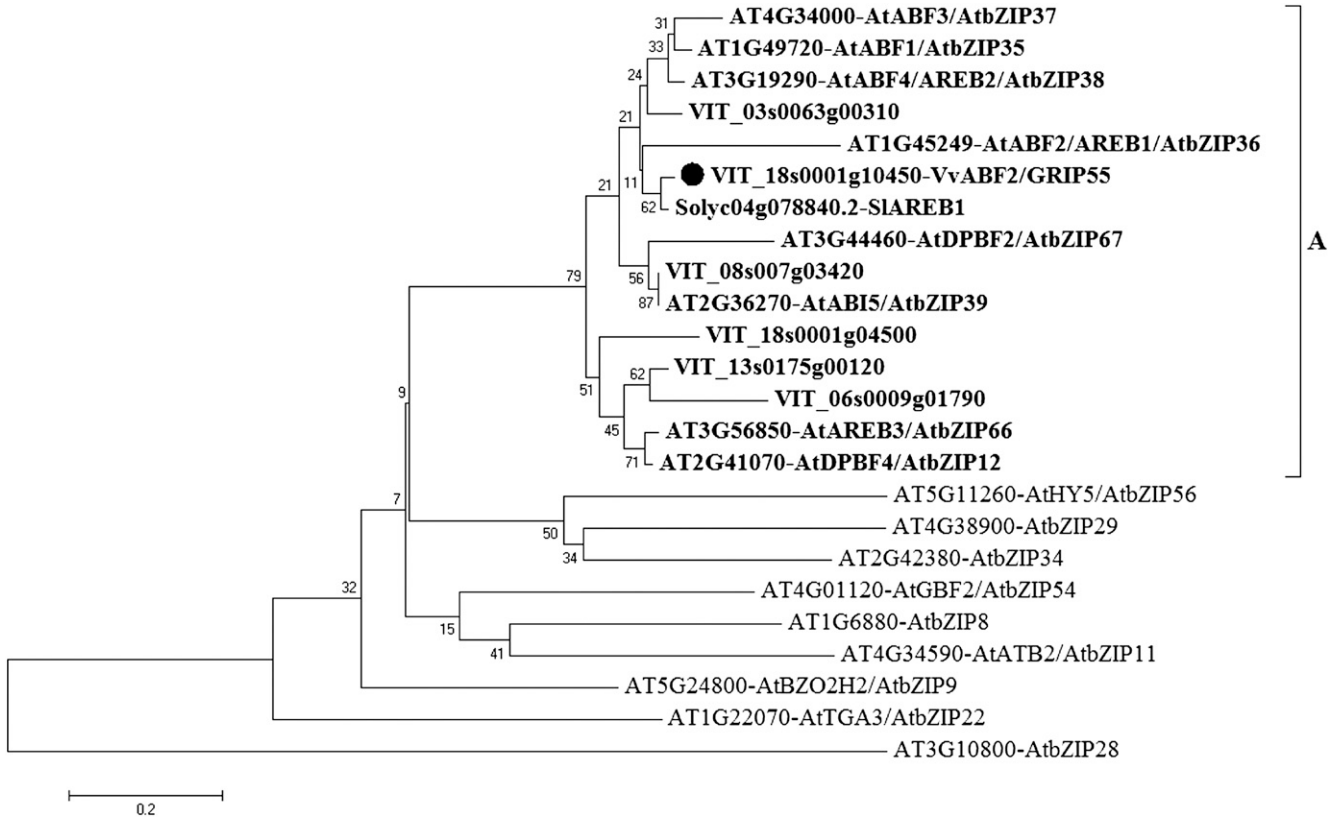

Figure 2. Phylogenetic analysis of VvABF2. The phylogenetic tree represents VvABF2 (black circle) and its orthologs (boldface) from the A subgroup of bZIP transcription factors in Arabidopsis (AT) and grape (VIT). The closest ortholog of VvABF2 from tomato (SlAREB1; Bastías et al., 2011) and representative Arabidopsis bZIP transcription factors from other bZIP subgroups (subgroup G, AtGBF2; H, AtHY5; I, AtbZIP29; E, AtbZIP34; D, AtTGA3; S, AtATB2; B, bZIP28; and C, AtBZO2H2) are also reported according to Jakoby et al. (2002) and Corrêa et al. (2008). The gene identifiers as well as synonyms (published names) are given. Multiple sequence alignments were generated using Clustal Omega (Sievers et al., 2011) on full-length proteins as implemented by MEGA software, version 5.0 (Tamura et al., 2011). The phylogenetic tree was constructed by neighbor joining with complete deletions as implemented by MEGA. Reliability values at each branch represent bootstrap samples (2,000 replicates).

this prediction, a GFP was fused in frame to the C terminus of VvABF2, and the resulting protein was expressed in tobacco (*Nicotiana tabacum*) mesophyll protoplasts under the control of the cauliflower mosaic virus 35S promoter. Unlike the GFP control, in which green fluorescence was seen throughout the cell, green fluorescence from VvABF2-GFP was only detected in the nucleus (Fig. 4A), consistent with a putative role of this protein in the control of transcription. In order to examine the transactivation ability of *VvABF2*, *GUS* reporter gene assays were performed by transient expression in tobacco protoplasts. The promoters of three known ABA up-regulated genes were selected: *VvLEA* (*V. vinifera LATE EMBRYOGENESIS ABUNDANT*; VIT_08s0007g04240), *VvNAC* (*V. vinifera NO APICAL MERISTEM* [*NAM*], *ARABIDOPSIS TRANSCRIPTION ACTIVATION FACTOR* [*ATAF*], *CUP-SHAPED COTYLEDON* [*CUC*]; VIT_19s0014g03290), and *VvBenzoR* (Vitis vinifera Benzodiazepine Receptor; VIT_07s0005g00140; Wang et al., 2011) and fused to the GUS reporter gene. Quantitative GUS expression analysis was assessed by using these constructs and VvABF2 as an effector protein in protoplasts treated or not with 20 μM ABA (Fig. 4B). All promoters tested displayed enhanced activities upon ABA treatment. Interestingly, coexpression of *VvABF2* significantly increased all promoter activities in the presence or absence of ABA (Fig. 4B). As multiple ABRE motifs were found in the promoter regions of these three VvABF2 putative target genes (Supplemental Table S1), the direct binding of VvABF2 was tested by electrophoretic mobility shift assay. Our results showed that VvABF2 binds specifically to these promoter regions, but only when ABRE motifs are present (Supplemental Fig. S1, A and C). Indeed, in the absence of these promoter elements, VvABF2 failed to bind to the promoter sequence (Supplemental Fig. S1B). Taken together, these results indicate that VvABF2 can recognize the ABRE sequences in the target promoters and activate these in response to ABA, under which activation levels were strongly enhanced. Therefore, VvABF2 is a positive

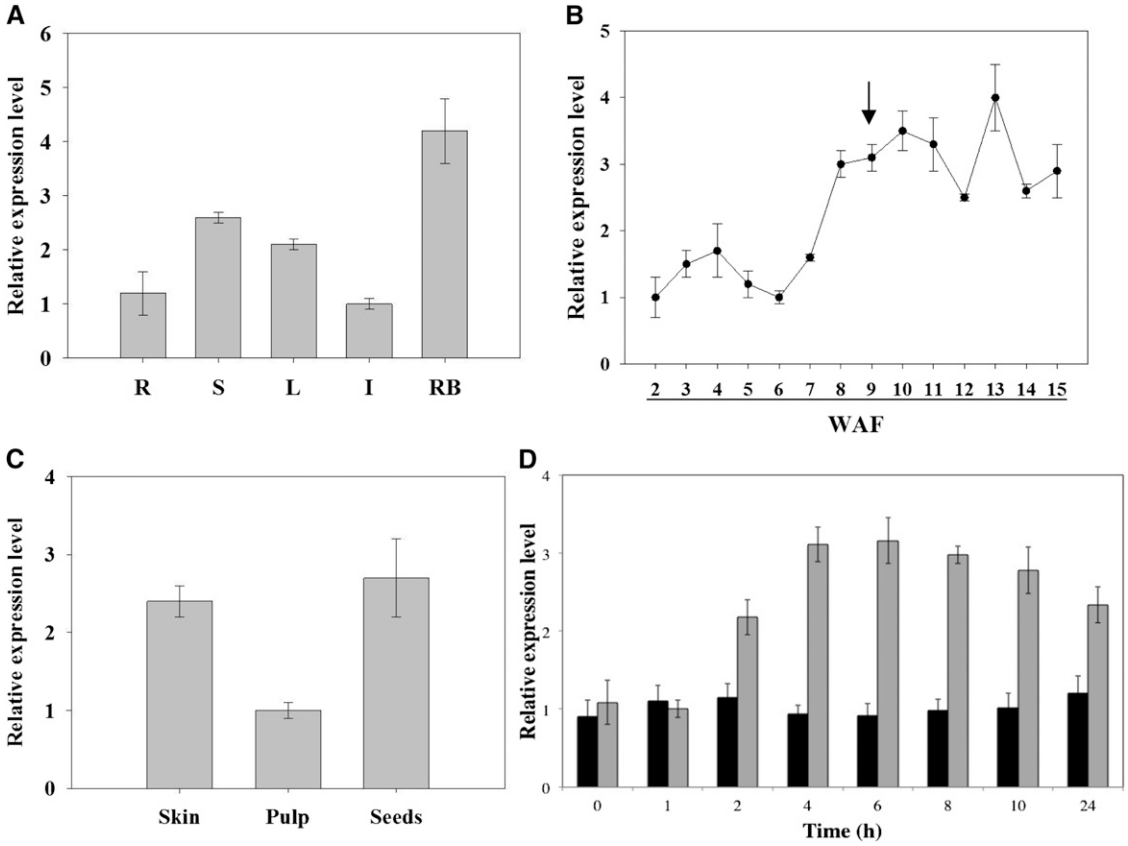

Figure 3. Quantitative real-time RT-PCR analysis of *VvABF2* expression patterns in grapevine cv Cabernet Sauvignon plants and ABA-treated cells. A, *VvABF2* expression in grapevine organs: roots (R), stems (S), leaves (L), inflorescences (I), and ripening berries at 11 WAF (RB). Error bars were calculated as SD for three independent experiments. Gene expression was normalized with *VvEF1γ* (*V. vinifera ELONGATION FACTOR1*; Q9SPF8). B, *VvABF2* expression at different stages of berry development, from 2 WAF to mature berries at 15 WAF. The arrow indicates the véraison stage. Error bars were calculated as the SD for four replicates from two independent experiments (summer 2006 and 2009). Gene expression was normalized with *VvEF1γ*. C, *VvABF2* expression in different tissues from ripening berries at 11 WAF. Error bars were calculated as the SD for three independent experiments. Gene expression was normalized with *VvEF1γ*. D, *VvABF2* transcript accumulation in cv Cabernet Sauvignon suspension cells treated with 20 μM ABA (gray bars) or with the same amount of ethanol (control; black bars). Error bars were calculated as SD for three independent experiments. Gene expression was normalized with *VvEF1γ*.

regulator modulating downstream ABA signaling pathways.

Production of Transgenic Cell Lines Overexpressing *VvABF2*

Transgenic grape cells overexpressing *VvABF2* were produced using a 35S-driven *VvABF2* construct. After stabilization of the cell suspension by subculture in glycerol-maltose-naphthoxyacetic acid culture medium supplemented with paromomycin and cefotaxime, the expression of *VvABF2* was tested by real-time RT-PCR using *VvABF2*-specific primers. In cells expressing the 35S::VvABF2 construct, *VvABF2* transcript accumulated six times more than in cells expressing the empty vector (Fig. 5). A 1-h ABA treatment (20 μM) of control cells dramatically stimulated *VvABF2* expression. ABA also further stimulated *VvABF2* expression in transgenic cells, but to a lesser extent (Fig. 5).

Transcriptomic Analysis of *VvABF2*-Overexpressing Cell Lines upon ABA Supply

Total RNAs extracted from 41B cells treated or not with 20 μM ABA for 1 h were hybridized with 60-mer oligoarrays bearing a set of probes for 29,582 unigenes (NimbleGen Gene Expression 12x135K Arrays). Analysis of differentially expressed genes was performed using a 2-fold expression change and an adjusted $P < 0.05$ (with false discovery rate correction) as a cutoff from three independent experiments. Differential gene expression analysis was assessed through microarray data comparison of three different experimental conditions: ABA-treated versus untreated control cell lines (condition 1), untreated *35S*::*VvABF2* versus untreated control cell lines (condition 2), and ABA-treated *35S*::*VvABF2* versus untreated control cell lines (condition 3). The overlap in genes differentially expressed in these three conditions was depicted with a three-way Venn diagram (Fig. 6A). The results showed that 1,722

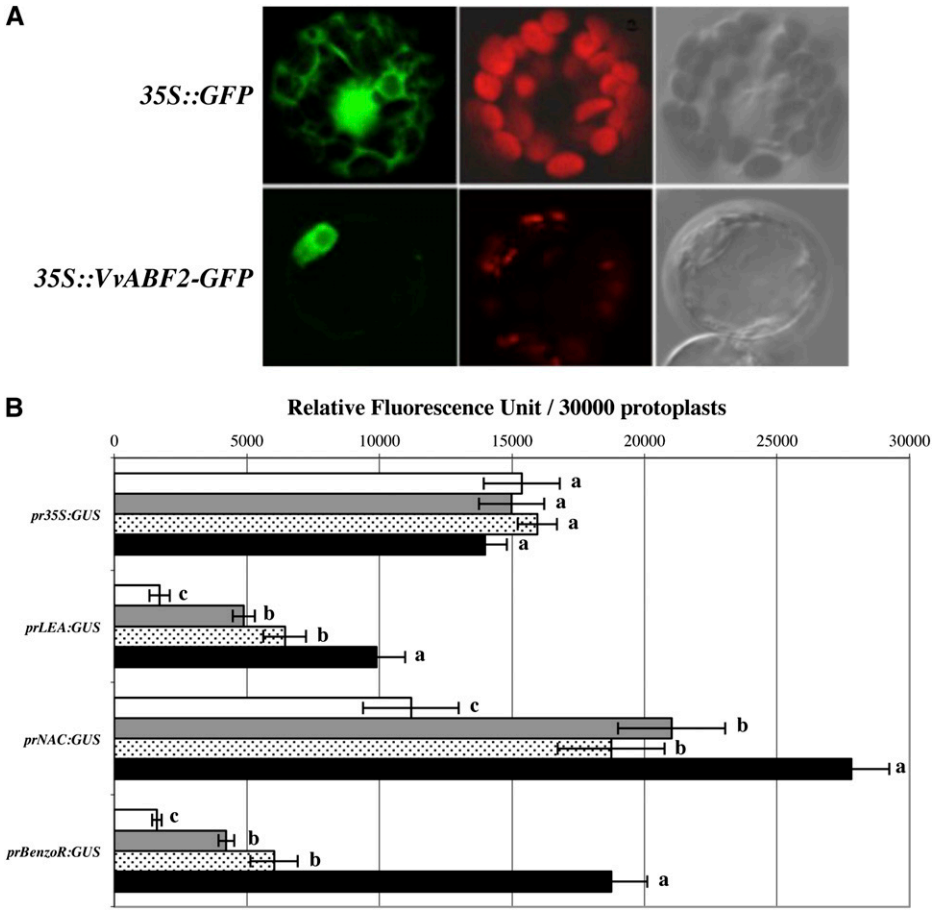

Figure 4. Subcellular localization and transactivation ability of *VvABF2*. A, Nuclear localization of the GFP-VvABF2 fusion protein in tobacco leaf protoplasts. These confocal microscopy images indicate, from left to right, protoplast sections analyzed for GFP fluorescence, the same sections analyzed for chloroplast autofluorescence, and the transmission light image from the same protoplast sections. B, Promoter activation by VvABF2 of selected ABA-regulated genes (Wang et al., 2011), *VvLEA* (VIT_08s0007g04240), *VvNAC* (VIT_19s0014g03290), and *VvBenzoR* (VIT_07s0005g00140), in tobacco protoplasts. *pr35S::GUS* was used as a positive control. White bars indicate GUS activity without additional construct or treatment, gray bars indicate GUS activity after transformation with the *35S::VvABF2* construct, dotted bars indicate GUS activity after 20 μM ABA treatment, and black bars indicate GUS activity after transformation with the *35S::VvABF2* construct coupled with 20 μM ABA treatment. Data from three independent experiments were pooled and analyzed. Error bars indicate SD. Statistical significance was assessed by one-way ANOVA followed by Tukey's honestly significant difference post-hoc test ($P \leq 0.05$).

genes were differentially expressed in at least one of the three conditions. More specifically, ABA treatment of control cell lines (condition 1) regulated 569 (1.9%) of the 29,582 unigenes represented on the microarray slide. Among these, 424 (74.5%) were up-regulated and 145 (25.5%) were down-regulated. In the absence of ABA treatment, overexpression of *VvABF2* led to the differential expression of 662 (2.2%) genes when compared with the control (condition 2). Three hundred thirty-eight (51%) genes were up-regulated and 324 (49%) were down-regulated (Fig. 6A). Treatment of *35S::VvABF2* transgenic cells with ABA resulted in the differential expression of 1,370 (4.6%) genes when compared with the untreated control cells (condition 3). Eight hundred eighty-one (64.4%) genes were up-regulated and 489 (35.6%) were down-regulated (Fig. 6A). The comparison of all three conditions showed that only 10 genes were commonly affected. Four hundred forty-one genes were common between conditions 2 and 3, and 438 genes were common between conditions 1 and 3. Moreover, 501 genes were specific for *35S::VvABF2* cells treated with ABA (condition 3; Fig. 6A).

The differentially expressed genes identified in our experiments were clustered based on expression ratios from conditions 1, 2, and 3 into 10 clusters of specific expression profiles (Fig. 6B). The clusters A, D, and J contained genes regulated by ABA in both control and *VvABF2*-overexpressing cells. Genes were up-regulated by ABA in clusters A and D and down-regulated in cluster J. Clusters B, E, G, and I included genes affected by *VvABF2* overexpression (up-regulated [B and E] and down-regulated [G and I]) independently of the presence of ABA. Clusters C, F, and H contained genes regulated by

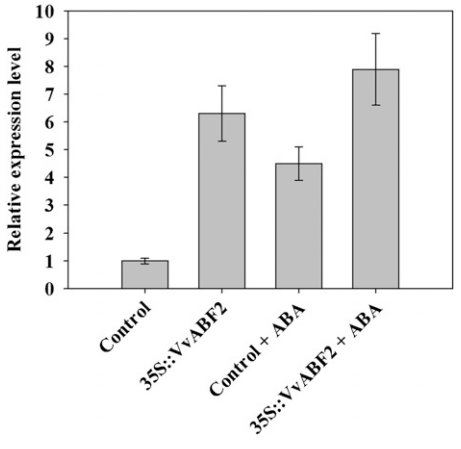

Figure 5. Relative expression level of *VvABF2* in transgenic grape 41B cells. *VvABF2* transcript level was assessed by quantitative real-time PCR in control (pFB8 empty vector) and *VvABF2*-overexpressing (*35S::VvABF2*) 41B cell lines, treated or not with 20 μM ABA for 1 h. Gene expression was normalized with *VvEF1*γ. Data are means of three independent experiments, and error bars are SD.

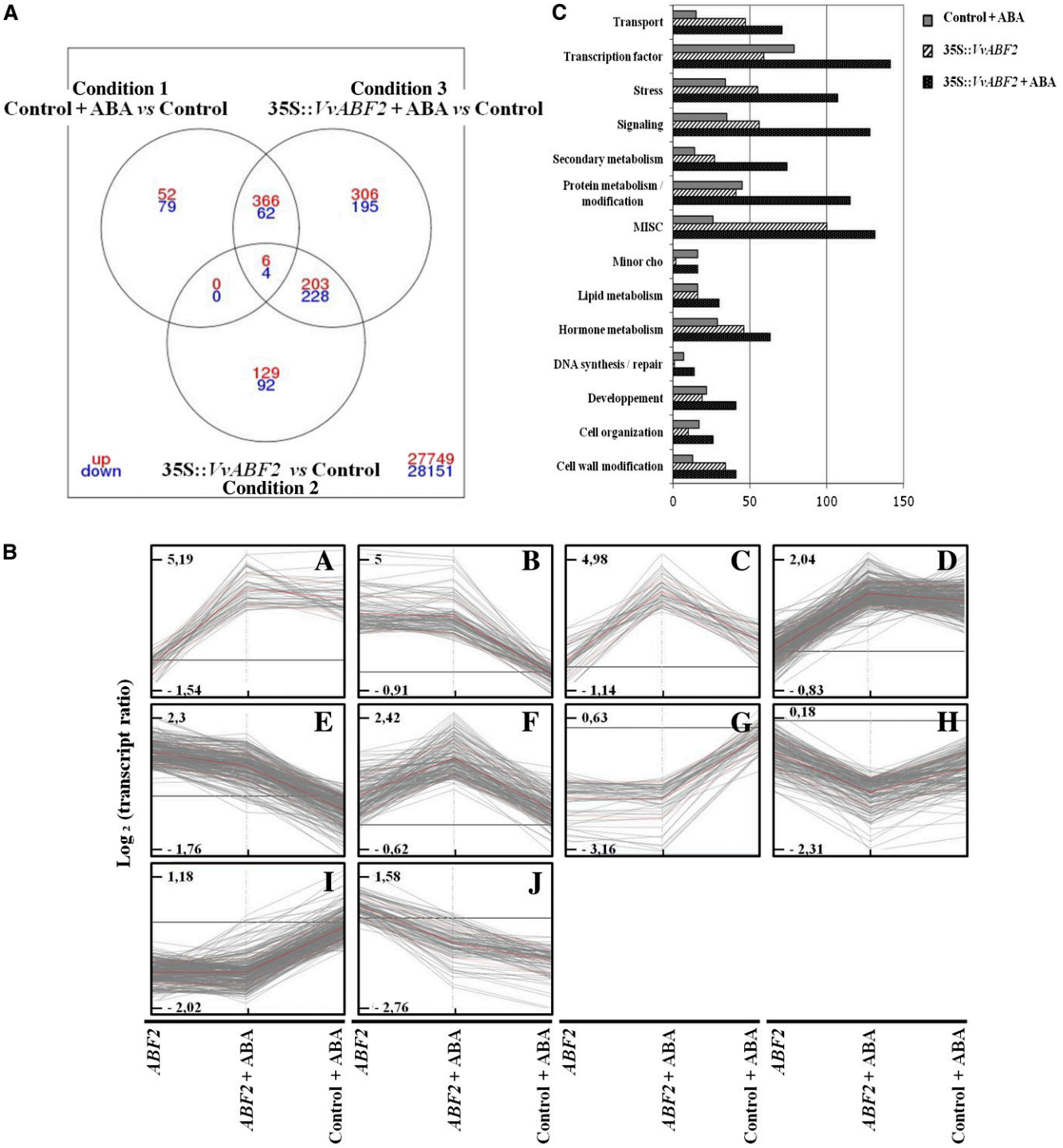

Figure 6. Overlap, expression profile clustering, and functional categorization of the 1,722 differentially expressed genes in the three experimental conditions. A, Three-way Venn diagram showing the overlap of differentially expressed genes in the three experimental conditions: condition 1 (control + ABA versus control), condition 2 (*35S::VvABF2* versus control), and condition 3 (*35S::VvABF2* + ABA versus control). B, Ten clusters have been created using MapMan (Thimm et al., 2004) on transcript ratios for the 1,722 differentially expressed genes under condition 1, condition 2, and condition 3. C, Classification of the 1,722 differentially expressed genes within selected MapMan ontology classes. The *x* axis indicates the number of genes within the different functional categories (*y* axis) for each condition: condition 1, condition 2, and condition 3. MISC, Miscellaneous.

ABA and/or ABF2. Clusters C and F encompassed genes significantly stimulated in *35S::VvABF2* cells treated with ABA, whereas cluster H corresponded to genes that were repressed in the same condition. Thus, clusters C, F, and H contained genes regulated by ABA in a VvABF2-dependent manner.

To functionally classify the 1,722 genes differentially expressed in all three types of conditions, we also performed an analysis using MapMan functional categories (Thimm et al., 2004; Fig. 6C). This classification suggested that most transcripts were linked to "secondary metabolism," "hormone metabolism," "protein metabolism," "transport," "signaling," "stress," and "transcription" when cells were supplemented with ABA (Fig. 6C). Among these ABA-stimulated groups, overexpression of *VvABF2* led to the further enrichment of most categories and, more particularly, of the groups linked to secondary metabolism (enrichment factor 5.5×), transport (4.5×), signaling (3×), stress (3×), and cell wall modification (3×).

The more altered biological functions with statistical significance among the 1,722 genes differentially expressed were identified using a MapMan Wilcoxon test (Table I). In condition 1, genes involved in calcium signaling ($P = 4.4 \times 10^{-16}$), minor CHO (carbohydrate) metabolism ($P = 3.19 \times 10^{-10}$), AP2/ERF (for APETALA2/Ethylene Responsive Element Binding Factor) transcription factor family proteins ($P = 4.44 \times 10^{-10}$), and phenylpropanoid metabolism ($P = 5.1 \times 10^{-9}$) were among the highest differentially expressed genes (Tables I and II). These genes followed the expression profile of cluster A or D (Fig. 6B). In addition, other modulated genes previously reported to exhibit ABA-regulated expression in grape berry (Koyama et al., 2010) and other plant species (Seki et al., 2002; Rabbani et al., 2003; Buchanan et al., 2005; Matsui et al., 2008), such as LEA and biotic/abiotic stress-related proteins, were also identified (Table II). In condition 2, genes involved in secondary metabolism ($P = 1.1 \times 10^{-13}$), protein degradation and modification ($P = 4.7 \times 10^{-4}$ and 2.2×10^{-7}, respectively), hormone metabolism and signaling (auxin and ethylene; $P = 7.63 \times 10^{-6}$ and 4.1×10^{-7}, respectively), and cell wall degradation ($P = 9.3 \times 10^{-5}$) were significantly affected by overexpression of *VvABF2* (Table I). These genes, which characterize the effect of VvABF2 on grape cells, belong to cluster B, E, G, or I (Fig. 6B). Some of these genes are shown in Table III. Among these (Table III), numerous genes encode cell wall hydrolytic enzymes (e.g. pectinesterases and polygalacturonases), secondary metabolism enzymes (e.g. flavonoid-*O*-glycosyltransferase, isoflavone reductases, dihydroflavonol 4-reductase, and isoflavone 2′-hydroxylase), hormone metabolism- and signaling-related proteins (e.g. IAA-amido synthetase and Ser/Thr protein kinase BRASSINOSTEROID INSENSITIVE1 [BRI1]-like), protein degradation-related proteins (e.g. F-box protein), sugar transport- and metabolism-related proteins (e.g. hexose transporter and invertase), transport-related proteins (e.g. multidrug and toxic compound extrusion [MATE], amino acids, and potassium transporter), transcription factors (e.g. ERF, MYELOBLASTOSIS [MYB], basic HELIX-LOOP-HELIX [bHLH], and HOMEODOMAIN LEUCINE ZIPPER), and stress-related proteins (e.g. pathogenesis-related [PR] protein, endochitinases, and peroxidases). Finally, in condition 3, the clustering into functional categories showed that genes involved in the synthesis of secondary metabolites were dramatically affected ($P < 1 \times 10^{-20}$). The enrichment was also observed for genes (Table I) corresponding to calcium signaling ($P < 1 \times 10^{-20}$), stress ($P < 1 \times 10^{-20}$), hormone metabolism and signaling (auxin and ethylene; $P = 2.5 \times 10^{-8}$ and 1.77×10^{-8}, respectively), and WRKY transcription factor ($P = 1.25 \times 10^{-7}$). Clusters C, F, and H contained genes affected by ABA in VvABF2-overexpressing cells (Fig. 6B; Table IV). The transcript abundance of key enzymes involved in secondary metabolism and, more particularly, in the first steps of the phenylpropanoid pathway was considerably increased in condition 3. These enzymes correspond to phenylalanine ammonia lyase (PAL) and cinnamate 4-hydroxylase. Interestingly, several stilbene synthase genes followed a similar expression pattern (Table IV). Numerous genes encoding proteins related to hormone

Table I. *P values of selected categories identified by the MapMan Wilcoxon test to be significantly different from all other differentially expressed genes*

Category Name	*P* Value, Condition 1 (Control + ABA Versus Control)	*P* Value, Condition 2 (*35S::VvABF2* Versus Control)	*P* Value, Condition 3 (*35S::VvABF2* + ABA Versus Control)
Calcium signaling	4.4×10^{-16}	0.4×10^{-2}	$<1–10^{-20}$
Secondary metabolism			
Phenylpropanoids/lignins	5.1×10^{-9}	6.8×10^{-8}	6.8×10^{-9}
Flavonoids/stilbenes	1.1×10^{-4}	1.1×10^{-13}	$<1–10^{-20}$
Minor CHO metabolism	3.2×10^{-10}	1.9×10^{-4}	1.3×10^{-8}
AP2-ERF transcription factor	4.4×10^{-10}	5.5×10^{-3}	6.6×10^{-11}
WRKY transcription factor	9.3×10^{-5}	8.7×10^{-2}	1.2×10^{-7}
Cell wall degradation	1.3×10^{-1}	9.3×10^{-5}	4×10^{-5}
Protein degradation/proteasome 26S	4.2×10^{-2}	4.7×10^{-4}	1.1×10^{-3}
Auxin metabolism and signaling	8.4×10^{-2}	7.63×10^{-6}	2.5×10^{-8}
Ethylene metabolism and signaling	2.2×10^{-5}	4.1×10^{-7}	1.77×10^{-8}
Protein modification/kinase	1.3×10^{-3}	2.2×10^{-7}	7.1×10^{-4}
Stress	1.6×10^{-5}	1.3×10^{-4}	$<1–10^{-20}$

Table II. *Selected ABA-induced genes from clusters A and D associated with minor CHO metabolism, signaling, flavonoid metabolism, stress, development, and transcription functional categories*

Ratio values are presented with $P < 0.05$.

Gene Name	Locus Name	Ratio, Condition 1 (Control + ABA Versus Control)	Ratio, Condition 2 (*35S::VvABF2* Versus Control)	Ratio, Condition 3 (*35S::VvABF2* + ABA Versus Control)
Minor CHO metabolism				
Raffinose synthase	VIT_17s0000g08960	5	1.2	4
Trehalose-6-phosphate synthase	VIT_11s0037g00720	2.2	1	2.1
Signaling				
PP2C	VIT_00s0179g00140	4	1	4
	VIT_11s0016g03180	4	1	3
SNF1-related protein kinase	VIT_03s0038g04580	3	1.1	4
Calmodulin-stimulated protein kinase	VIT_18s0001g06180	5	1.1	6
Calmodulin	VIT_17s0000g02480	2.3	0.9	2.3
	VIT_01s0010g02950	3	0.8	3.5
	VIT_16s0100g00620	2	0.9	2
Secondary metabolism				
Anthocyanidin 3-*O*-glucosyltransferase	VIT_19s0085g00750	4	1	3
Flavanone 3-hydroxylase	VIT_18s0001g03510	4	1	4
Stress				
Heat stress transcription factor	VIT_00s0179g00150	10	0.9	10
Pathogenesis-related protein	VIT_06s0004g04010	3.5	0.8	3.5
17.2-kD class II heat shock protein	VIT_09s0002g00640	2.5	1	2.5
Aquaporin TIP1-1	VIT_08s0007g04780	3.2	1.2	3.3
Desiccation-related protein	VIT_05s0077g00610	2.1	1	2.1
Senescence-associated protein	VIT_00s2814g00010	3	1.1	3
Development				
Late embryogenesis abundant protein	VIT_13s0067g01250	5	1.2	5
	VIT_08s0007g06420	3	1.1	3
	VIT_16s0115g00170	4.5	1.1	5
Transcription				
AP2/ERF transcription factor	VIT_07s0031g00720	34	0.7	34
	VIT_04s0008g02230	5	0.9	6
	VIT_15s0046g00310	2.5	1.1	3
DREB protein	VIT_18s0001g13320	7	1	7
	VIT_13s0067g01960	2.3	1	2.3
NAC transcription factor	VIT_19s0014g03290	10	0.9	10
	VIT_02s0236g00100	8	0.9	8
ABI5	VIT_03s0063g00310	2.3	1	2.3

metabolism and signaling (e.g. 1-aminocyclopropane-1-carboxylate oxidase, GA receptor, and GA 2-oxidase), protein degradation (e.g. F-box protein, ubiquitin-protein ligase, and RING-H2 finger protein), and transport (e.g. ATP-binding cassette [ABC] transporter G family member and Glu receptor) were also strongly increased in the same condition. Finally, the transcript abundance of transcription factors (e.g. dehydration-responsive element-binding protein [DREB], WRKY, and MYB) and stress-related proteins (e.g. PR proteins, endochitinases, peroxidases, and receptor-like kinase) was also increased in *VvABF2*-overexpressing cells supplied with ABA (Table IV).

VvABF2 Overexpression Stimulates ABA Induction of Stilbene Production

Stilbenes, resveratrol, and its derivatives such as trans-piceid (a glycosylated form of resveratrol) represent some of the major forms of phytoalexins in grapevine (Pezet et al., 2004). They are important for plant defense, and resveratrol also has beneficial effects on human health (Bradamante et al., 2004; Hofseth et al., 2010; Szkudelska and Szkudelski, 2010). Stilbene production in grapevine transgenic cell suspensions was monitored after the addition of 20 μM ABA. Before treatment, control and *35S::VvABF2* transgenic cells secreted different amounts of each stilbene in the culture

Table III. *Selected VvABF2-regulated genes from clusters B, E, G, and I associated with cell wall metabolism, secondary metabolism, hormonal metabolism and signaling, protein degradation, sugar metabolism and transport, transcriptional regulation, transport, and stress response functional categories*

Ratio values are presented with $P < 0.05$.

Gene Name	Locus Name	Ratio, Condition 1 (Control + ABA Versus Control)	Ratio, Condition 2 (*35S::VvABF2* Versus Control)	Ratio, Condition 3 (*35S::VvABF2* + ABA Versus Control)
Cell wall metabolism				
Pectinesterase	VIT_18s0001g12670	1	11	11
	VIT_16s0022g00710	1	8	8
	VIT_03s0038g04740	1	5	5
Polygalacturonase	VIT_14s0066g01060	1	5.9	3.5
Rhamnogalacturonate lyase	VIT_00s0346g00030	1	3.7	4
Expansin	VIT_07s0005g02310	0.7	3	2
Endoglucanase	VIT_02s0087g00930	0.8	0.25	0.25
	VIT_14s0036g01040	1	0.25	0.25
Secondary metabolism				
Flavonoid 5,3-*O*-glucosyltransferase	VIT_18s0041g00900	0.8	7	5
	VIT_18s0041g00800	1	5	5
	VIT_18s0041g00970	0.8	3.5	3
Isoflavone reductase	VIT_02s0033g00260	1	3.1	3.5
Dihydroflavonol 4-reductase	VIT_02s0025g01260	1	3	2.9
	VIT_01s0011g03480	1	0.3	0.3
Isoflavone 2′-hydroxylase	VIT_09s0002g06450	1	3.2	2
Laccase	VIT_18s0164g00170	1.1	3.3	2
Hormonal metabolism and signaling				
IAA-amido synthetase	VIT_12s0134g00230	0.9	12.5	8.8
	VIT_01s0150g00300	0.7	3.7	3
AUXIN/IAA family protein	VIT_05s0020g04690	0.9	6.5	4.6
SAUR family protein	VIT_03s0038g01220	0.9	5.8	4.3
PIN family protein	VIT_11s0052g00440	0.4	4.9	2.3
9-cis-Epoxycarotenoid dioxygenase	VIT_02s0087g0093	0.9	0.25	0.3
Ser/Thr protein kinase BRI1-like	VIT_16s0013g01500	1.1	3	3.7
	VIT_00s0316g00010	0.9	3.2	3.2
Protein degradation				
F-box protein	VIT_01s0011g0122	1	11	5
Ubiquitin family protein	VIT_14s0219g00210	1	4.6	4.9
Sugar transport and metabolism				
Vacuolar invertase	VIT_16s0022g00670	1	3.1	3
Hexose carrier protein (HT4)	VIT_16s0013g01950	1	3	3
Polyol transporter	VIT_03s0063g02250	1	3.5	3.2
Transport				
Amino acid permease	VIT_18s0001g01850	1	3.6	3
Vacuolar amino acid transporter	VIT_19s0027g01890	0.6	3	4
Peptide transporter PTR2	VIT_18s0001g11280	1	4	3.5
MATE efflux family protein	VIT_13s0064g00940	1	5.5	5.1
	VIT_16s0100g00460	0.7	10	4
Potassium transporter	VIT_01s0011g03020	1	7.2	6
Potassium channel KAT3	VIT_04s0008g04510	1.2	3	3
Aquaporin PIP1.1	VIT_13s0067g00220	1	3.5	5
ABC transporter family protein	VIT_19s0085g00060	1	0.01	0.01
MATE efflux family protein	VIT_08s0056g00870	1	0.3	0.3
GDP-Man transporter	VIT_16s0022g00370	0.6	0.01	0.01
Transcription				
Ethylene-responsive transcription factor	VIT_10s0003g00590	0.8	6	5
Homeobox-Leu zipper protein HB40	VIT_04s0023g01330	1.1	8	10
MYB transcription factor MYB36	VIT_11s0016g02780	0.8	4	5
Transcription factor bHLH68	VIT_11s0016g03560	1	6	4.5
Dof zinc finger protein	VIT_07s0255g00020	1	4.5	4
	VIT_10s0003g00040	0.9	4.5	3
bHLH transcription factor	VIT_00s0274g00070	0.8	0.3	0.3
	VIT_07s0205g00190	1	0.3	0.4
MYB transcription factor	VIT_09s0070g00410	0.9	0.2	0.3

(*Table continues on following page.*)

Table III. (*Continued from previous page.*)

Gene Name	Locus Name	Ratio, Condition 1 (Control + ABA Versus Control)	Ratio, Condition 2 (*35S::VvABF2* Versus Control)	Ratio, Condition 3 (*35S::VvABF2* + ABA Versus Control)
Homeobox-Leu zipper protein KNAT1	VIT_18s0001g08380	0.8	0.2	0.2
Stress response				
PR protein	VIT_03s0088g00700	0.8	32	26
	VIT_08s0040g02170	1.1	4.5	4.5
	VIT_13s0147g00150	1.4	4	4.5
	VIT_18s0041g02190	1.1	0.3	0.3
Endochitinase	VIT_05s0094g00280	1.3	21	20
	VIT_16s0050g02210	0.9	4	3.6
Peroxidase	VIT_07s0104g01100	1.1	5	3.6
	VIT_10s0116g00340	1	5	4.2
	VIT_11s0016g05280	0.6	0.3	0.2
Accelerated cell death	VIT_14s0081g00350	1	3	3.3
Protein kinase resistance-like	VIT_12s0028g01850	0.8	0.2	0.2

medium. Indeed, in control cells, trans-resveratrol was present at concentrations representing 57.6% of its amount in *VvABF2* transgenic cells. By contrast, in nontreated *VvABF2*-overexpressing cells, trans-piceid only represented 56% of that present in nontreated control cells (Fig. 7). However, the total amount of stilbenes is similar for both conditions (Supplemental Fig. S2).

After the addition of ABA to control cell suspensions, trans-resveratrol strongly accumulated in the culture medium (5.6-fold more), whereas the trans-piceid amount was maintained (Fig. 7). By contrast, in the culture medium of *35S::VvABF2* cell suspensions, ABA treatment increased both trans-resveratrol and trans-piceid contents (Fig. 7). Indeed, trans-resveratrol and trans-piceid contents were multiplied by maximal factors of 6.2 and of 2.5, respectively. Finally, ABA treatment increased the total stilbene amount by 2.7-fold in control cells and by 4.1-fold in the transgenic cells (Supplemental Fig. S2).

Phenotype of Tomato Fruits Overexpressing VvABF2

To investigate the involvement of *VvABF2* in fruit development and ripening processes, transgenic tomatoes overexpressing *VvABF2* were produced. The full-length coding region of *VvABF2* was cloned downstream of the cauliflower mosaic virus *35S* promoter and used for transformation. Five independent *35S::VvABF2* transgenic plants were selected for their successful integration of the transgene (Supplemental Fig. S3). *VvABF2* overexpression did not result in apparent developmental defects. Flowers and fruits developed normally until the turning stage, with kinetics comparable to the control plants. By contrast, after the turning stage and at the later ripening stages, fruits from all *35S::VvABF2* lines exhibited dramatic phenotypes. Indeed, as soon as 20 d after turning, the firmness of the tomatoes from *VvABF2*-overexpressing lines was strongly reduced in comparison with the control fruits. This reduction in firmness is illustrated in Supplemental Figure S3, showing the phenotype observations and the Durofel indices that were obtained for the control and the *VvABF2*-overexpressing fruits 20 or 50 d after turning.

DISCUSSION

Ripening of nonclimacteric fruit is usually associated with ABA, a phytohormone that gradually accumulates from the onset of ripening (Coombe, 1992; Giribaldi et al., 2010; Koyama et al., 2010). Two different ABA receptors from strawberry (*Fragaria* spp.), FaCHLH/ABAR (for *Fragaria ananassa* H subunit of magnesium chelatase/ABA receptor; Jia et al., 2011) and FaPYR1 (Chai et al., 2011), and one receptor from grape, VvPYL1 (Li et al., 2012), were recently described as positive regulators of fruit ripening in response to ABA. In this context, AREB/ABF bZIP transcription factors that act as regulators of ABA and stress responses in plants are good candidates for the regulation of ABA-mediated fruit ripening. Moreover, a possible role of AREB/ABF transcription factors has been reported for the ripening climacteric fruits (Bastías et al., 2011).

This work gives further insight into the role of this class of transcription factor during the ABA-mediated ripening of grape berries. In this context, we isolated and characterized VvABF2, previously known as GRIP55 (VIT_18s0001g10450, Q9M4H1). VvABF2 belongs to group A of bZIP transcription factors, which are involved in ABA and abiotic stress signaling (Choi et al., 2000; Uno et al., 2000; Amir Hossain et al., 2010). *VvABF2* was ubiquitously expressed in different grape organs, and its transcript accumulated just before the onset of grape berry ripening (Fig. 3), when ABA concentrations increase (Coombe, 1992). These data fit well with the observation that *VvABF2* expression was regulated by ABA (Fig. 3D) and underline a putative role for this transcription factor in influencing ABA-regulated grape berry ripening processes. The nuclear localization of VvABF2 (Fig. 4A) and its ability to transactivate selected promoters (Fig. 4B) are consistent with its function as a transcriptional activator. Additionally, the transactivation experiments performed in protoplasts further

Table IV. *Selected genes transcriptionally regulated in VvABF2-overexpressing cells treated by ABA, belonging to clusters C, F, and H, and associated with secondary metabolism, hormonal metabolism and signaling, protein degradation, transport, transcriptional regulation, and stress response functional categories*

Ratio values are presented with $P < 0.05$.

Gene Name	Locus Name	Ratio, Condition 1 (Control + ABA Versus Control)	Ratio, Condition 2 (*35S::VvABF2* Versus Control)	Ratio, Condition 3 (*35S::VvABF2* + ABA Versus Control)
Secondary metabolism				
Stilbene synthase	VIT_10s0042g00840	1.2	2.8	5.5
	VIT_16s0100g00780	1.5	2.2	6.5
	VIT_16s0100g01020	1.7	1.3	13
	VIT_16s0100g00960	1.6	1.3	8
Cinnamate 4-hydroxylase	VIT_11s0078g00290	1.8	1.3	7
	VIT_11s0065g00350	2	1.3	13
CAD	VIT_18s0122g00450	1.4	3.2	5
PAL	VIT_16s0039g01170	2	0.9	6
	VIT_16s0039g01320	2.1	0.9	5
	VIT_00s2508g00010	2.1	0.8	4.5
	VIT_08s0040g01710	1.6	0.9	3
Hormonal metabolism and signaling				
AUXIN/IAA family protein	VIT_11s0016g04490	1.8	1.5	3
1-Aminocyclopropane-1-carboxylate oxidase	VIT_18s0001g01840	1.1	12	19
GA receptor GID1	VIT_01s0011g03270	1.2	3.6	10
GA 2-oxidase	VIT_19s0140g00120	3.5	2.3	8
ABA 8′-hydroxylase	VIT_06s0004g05050	1.9	1.1	3
Cytokinin-*O*-glucosyltransferase	VIT_13s0019g03120	1.2	1.6	3.5
Ser/Thr protein kinase BRI1-like	VIT_09s0018g00780	1.3	1.3	4.5
Protein degradation				
F-box protein	VIT_00s1386g00020	1.1	1	22
	VIT_10s0116g01290	1.2	1	19
Ubiquitin-protein ligase	VIT_08s0105g00190	2.7	1	11
	VIT_18s0166g00190	1.2	0.8	3
Cupin superfamily protein	VIT_07s0005g04620	1	1.2	3
RING-H2 finger protein	VIT_05s0077g01970	2	0.8	3.7
Transport				
ABC transporter G family member	VIT_06s0061g01490	1.5	1.1	4
Glu receptor	VIT_05s0051g00780	1.5	1	3
Amino acid transporter	VIT_03s0038g03530	1.2	1.1	3

support the hypothesis of the involvement of VvABF2 in ABA-mediated signaling pathways. Indeed, VvABF2 was able to bind and transactivate several known ABA-regulated genes (*VvLEA*, *VvNAC*, and *VvBenzoR*), and its transcriptional activating role was further increased by ABA supply (Fig. 4B).

The function of VvABF2 and its role in ABA signaling were further analyzed by a transcriptomic approach comparing the response to ABA of transgenic grape cells that overexpressed *VvABF2* with that of control cells. Overexpression of *VvABF2* (condition 2) modulates the expression of many grape genes in the absence of ABA. Only a few (10) genes were common with those observed in ABA-treated control cells (condition 1). This might be the consequence of an ABA treatment (1 h) that was too short to affect all ABA-regulated genes in control cells. This hypothesis was strengthened by the fact that many of the VvABF2-regulated genes (condition 2) belong to families previously shown to be involved in ABA responses. This also indicates that the ectopic expression of *VvABF2* was sufficient to affect some of the ABA-dependent genes. In addition, ABA treatment of *35S::VvABF2* transgenic cells (condition 3) regulated many other genes, including 438 genes that were common with ABA-treated control cells (condition 1). This represents 77% of the total number of genes affected by the ABA treatment in the control cells (among which 65.4% were up-regulated and 11.6% were down-regulated). By comparison, ABA treatment of *35S::VvABF2*-overexpressing cells resulted in the modification of the expression of 32% of all the genes affected by VvABF2 overexpression (27.2% up-regulated and 4.8% down-regulated). These results indicated that the expression of some genes that follow an ABA-dependent pathway were mainly mediated by VvABF2, whereas others were specifically dependent on VvABF2 and independent of ABA treatment. The identification and in silico analysis of 1,344 bp of the *VvABF2* promoter region using the Genomatix suite of programs (Quandt et al., 1995) led to the identification of several putative cis-acting regulatory elements within the 5′ regulatory region of *VvABF2* (Supplemental Table S2). These transcription factor-binding sites were associated

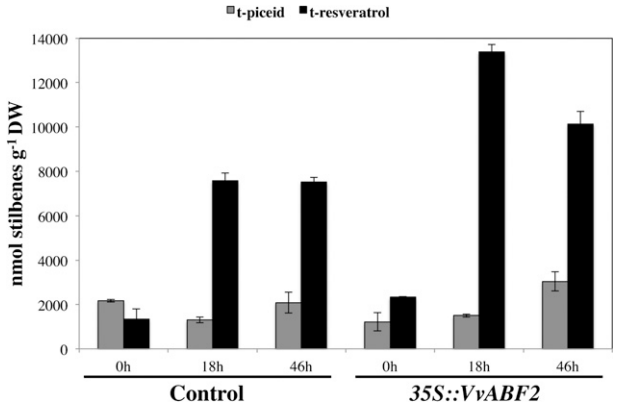

Figure 7. Time course of stilbene production (nmol g^{-1} dry weight [DW]) in the culture medium of control and *35S::VvABF2* transgenic cells supplied with 20 μM ABA. Total trans-piceid (gray bars) and trans-resveratrol (black bars) were measured in the extracellular medium. Values represent means ± SD of triplicate assays of one representative experiment out of three.

with plant development, hormonal regulation, and biotic and abiotic stress responses. Their presence occurred at different frequencies and are described in Supplemental Table S2. This result suggests the existence of both ABA-dependent and -independent pathways in the control of *VvABF2* expression.

ABA treatment of grape cells led to the regulation of many genes known to be ABA and stress related. For instance, genes specifically affected by the ABA treatment encode (1) proteins involved in osmotic stress, desiccation (raffinose synthase and LEA), and biotic stress (PR protein) responses, (2) proteins known to improve stress tolerance to abiotic stresses (AP2-DREB, bZIP, and NAC), and (3) members of ABA signaling (PP2C) and calcium signaling (calmodulin and calmodulin-stimulated protein kinase; Desikan et al., 2001; Yu et al., 2006; Nishimura et al., 2007; Nakashima et al., 2009; Park et al., 2009; Wang et al., 2011; Table II). Our results confirm data from the literature and validate these experiments.

As mentioned before, *VvABF2* overexpression can affect some ABA-dependent genes in the absence of ABA treatment (Table III). For instance, several transcripts encoding PR proteins such as endochitinases were strongly up-regulated in *VvABF2* transgenic cells. These genes are induced during berry development and ripening (Davies and Robinson, 2000; Pilati et al., 2007). Genes encoding potassium transporters and channels were also strongly up-regulated (Table III). These genes are known ABA targets that are also important for berry ripening (Davies et al., 2006). Indeed, together with sugar accumulation, K^+ influx might help the turgor-driven berry expansion (Davies et al., 2006), and the K^+ channel VvK1.1 may play a major role in K^+ loading into berry tissues, especially upon drought stress (Cuéllar et al., 2010, 2013).

Effects on Ripening-Associated Processes

ABA promotes sugar metabolism and accumulation in fleshy fruits (Yamaki and Asakura, 1991; Kobashi et al., 1999; Richings et al., 2000; Pan et al., 2005). In tomato, overexpression of *SlAREB1* stimulates hexose accumulation in correlation with increased expression of vacuolar invertases (Bastías et al., 2011). In *VvABF2*-overexpressing cells, the stimulated expression of vacuolar invertase and hexose transporter genes is consistent with this hypothesis (Table III).

The softening that accompanies the last stages of fleshy fruit ripening is typically attributed to changes in cell wall properties (Li et al., 2010). Several studies have underlined the role of ABA in enhancing softening in both tomato (Zhang et al., 2009b) and grapevine (Gambetta et al., 2010). Indeed, it was suggested by Koyama et al. (2010) that in grape berry, the modification of cell walls occurring under ABA treatment may be reflected by the differential transcript abundances of cell wall proteins and pectin-modifying enzymes. The high transcript levels of genes encoding cell wall hydrolytic enzymes (pectinesterase, polygalacturonase, and rhamnogalacturonase) in *VvABF2*-overexpressing cells suggest a role for VvABF2 in fruit softening (Table III). This hypothesis is further strengthened by the observation that in tomato fruits overexpressing *VvABF2*, the softening process is dramatically accelerated (Supplemental Fig. S3).

Responses to oxidative stress have been reported during fruit development in several species, including tomato (Jiménez et al., 2002), strawberry (Aharoni and O'Connell, 2002), and grape (Pilati et al., 2007). Reactive oxygen species (ROS) amounts are accurately controlled by both their production and the antioxidant systems. During grape berry development, Pilati et al. (2007) showed that oxidative processes and enzyme-mediated scavenging systems are activated during the ripening stage. In agreement with this work, our microarray data showed that both ROS production and scavenging systems were affected (Supplemental Table S4). Indeed, in at least one condition (in the presence of ABA and/or VvABF2), the expression of 30 genes involved in ROS production was significantly affected. These genes belong to the RBOH (respiratory burst oxidase homologue) and secretory peroxidase (class III) families that are responsible for the production of ROS and of some secondary metabolites leading to the synthesis of antifungal products or the reinforcement of cell walls. In the same conditions, the expression of more than 80 genes related to ROS scavenging systems was also altered. These include ascorbate and glutathione peroxidases, peroxiredoxins, thioredoxins, glutaredoxins, and glutathione *S*-transferases. The fine-tuned control of this gene network by ABA and/or VvABF2 may contribute to the regulation of the redox balance and to the production of some metabolites that are necessary for ripening.

The role of ABA in enhancing the synthesis of phenolic compounds during fruit ripening has been reported extensively (Ban et al., 2003; Jeong et al., 2004; Peppi et al., 2006; Wheeler et al., 2009; Gambetta et al., 2010). Our transcriptomic analysis revealed that ABA regulates some genes encoding secondary metabolism enzymes and that *VvABF2* overexpression does it as

well: for example, treatment of control cells with ABA up-regulated genes involved in flavonoid/anthocyanin biosynthesis (Table II). Among these, *flavanone 3-hydroxylase* was previously shown to be induced in berry skin after ABA treatment (Koyama et al., 2010). Likewise, *VvABF2* overexpression also led to the stimulation of this pathway (Table III). Indeed, the expression of genes encoding proteins involved in the biosynthesis of anthocyanins (*UDP-glucose:flavonoid 3-O-glucosyltransferase* and *dihydroflavonol reductase*) and isoflavones (*isoflavone hydroxylase* and *isoflavone reductase*) is modulated by VvABF2. Finally, treatment of *35S::VvABF2* cells by ABA also up-regulated secondary metabolism and, more particularly, the first steps of the phenylpropanoid pathway (PAL and cinnamic acid 4hydroxylase), the lignin biosynthesis pathway (cinnamyl alcohol dehydrogenase [CAD]), and the phytoalexin pathway (stilbene synthase). The strong up-regulation of these genes in ABA-treated *35S::VvABF2* cells by comparison with ABA-treated control cells suggests that their expression depends on both ABA and VvABF2. Measurements of stilbene production in *35S::VvABF2* transgenic cells corroborate our microarray data. Indeed, ABA treatment of control and VvABF2-overexpressing cells stimulated this production and particularly that of trans-resveratrol (Fig. 7). Therefore, trans-resveratrol secretion is stimulated by ABA in both control and *35S::VvABF2* cells (5.6- and 6.2-fold increases, respectively, when compared with non-treated cells). By contrast, trans-piceid amounts did not significantly change in ABA-treated control cells, whereas its amount was more than doubled in *35S::VvABF2* transgenic cells treated with ABA.

Overexpression of *VvABF2* also affected the accumulation of MATE transporter transcripts. Since this gene family is involved in the transport of flavonoids (Gomez et al., 2009; Zhao and Dixon, 2009), these data indicate that VvABF2 may also be involved in the regulation of flavonoid transport.

Effects on Genes Controlling Hormonal Balance

Hormone balance is important for fruit development and ripening. For example, the maintenance of precise amounts of cytokinins is needed to achieve fruit ripening (Mok, 1994). Low levels of free IAA (maintained by Gretchen Hagen3 [GH3]) are also needed to allow berry ripening (Böttcher et al., 2010, 2011), and ABA stimulates this conjugating enzyme (Seo et al., 2009). Ethylene plays an important role in climacteric fruit ripening (Giovannoni, 2007), and ABA is thought to control the ripening of climacteric fruit through the activation of ethylene biosynthesis (Zhang et al., 2009b). Although the role of ethylene in nonclimacteric fruits remains unclear, its involvement in grapevine berry ripening has been suggested (Chervin et al., 2004, 2008). Finally, brassinosteroids are positive hormonal regulators of berry ripening (Symons et al., 2006) that interact with other phytohormones (ABA and auxin) to achieve their biological function (Hardtke, 2007; Zhang et al., 2009). Our results suggest a fine-tuning of hormone amounts and signaling pathways by ABA and underline a putative role of VvABF2 in this control. Indeed, *VvABF2*-overexpressing cells accumulated transcripts of genes involved in hormone biosynthesis and/or signaling (Tables III and IV). Two sets of genes could be identified. The first one corresponded to genes stimulated by ABA in control cells, and their expression was further induced by *VvABF2* overexpression. The second set included genes that were already affected in *35S::VvABF2* cells and whose accumulation was further affected by ABA supply, including many genes involved in the control of hormone amounts and, more particularly, their biosynthesis (*9-CIS-EPOXYCAROTENOID DIOXYGENASE* and *1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE*) and catabolism (*GH3*, *GA 2-OXIDASE*, *ABA 8′-HYDOXYLASE*, and *CYTOKININ-O-GLUCOSYLTRANSFERASE*; Tables III and IV). Other genes corresponding to proteins involved in hormone signaling were also affected, including genes involved in hormone sensing (*Gibberellin Insensitive Dwarf1* [*GID1*] and *BRI1*) and transduction (*AUXIN/IAA* and *small auxin up RNA* [*SAUR*]).

The ubiquitin/26S proteasome pathway plays a key role in the perception and transmission of environmental and hormonal signals (Smalle and Vierstra, 2004; Liu and Stone, 2010; Antoni et al., 2011). In this work, the importance of this pathway in ABA signaling is highlighted by the response of several genes encoding F-box proteins and other components of the proteasome pathway in *35S::VvABF2* transgenic lines and after ABA supply (Table III).

Effects on Genes Involved in Stress Responses

Numerous transcription factors are regulated by ABA or ABA-regulated stresses, even though their specific roles in ABA signaling remain unknown (Nemhauser et al., 2006; Yamaguchi-Shinozaki and Shinozaki, 2006). ABA-regulated members of the DREB, WRKY, and MYB transcription factor families were up-regulated when *VvABF2* was overexpressed (Table III), reinforcing the role of this transcription factor in regulating ABA-mediated pathways. Thus, among the known homologs of the transcription factor genes identified, *AtWRKY28* and *AtWRKY40* are involved in oxidative stress, cold, high-salinity, and osmotic stress responses (Seki et al., 2002) and *AtWRKY33* and *AtWRKY53* are involved in hydrogen peroxide and ozone responses (Tosti et al., 2006; Vanderauwera et al., 2007). Additionally, it has been suggested that AtWRKY33 may regulate plant responses to both abiotic stress and ABA (Jiang and Deyholos, 2009). In addition, AtWRKY40 binds the promoters of several members of the AREB/ABF transcription factor subfamily, and its activity is inhibited by ABA perception by the magnesium-chelatase H subunit receptor (CHLH/ABAR; Shang et al., 2010).

Taken together, these data show that *VvABF2* overexpression not only activates new gene networks but mainly functions by exacerbating and/or modifying

existing networks related to ABA responses. Indeed, ABA treatment of *VvABF2*-overexpressing cells often led to an enhanced response compared with ABA treatment of control cells (Fig. 6B, clusters C, F, and H). This was particularly observed for genes involved in the phenylpropanoid pathway. Several genes encoding enzymes involved in the first step of this pathway (PAL and C4H) and several stilbene synthases were strongly induced. These data indicate that in the presence of ABA, VvABF2 is an important regulator of the phenylpropanoid pathway leading to stilbene biosynthesis. This result is consistent with data from the literature showing an up-regulation of several stilbene synthase genes paralleling an increase in resveratrol concentrations in ABA-treated berries (Koyama et al., 2010). In addition, VvABF2 seems to affect lignin biosynthesis by the stimulation of CAD and laccase genes (Tables III and IV). The induction of lignin biosynthetic genes by ABA was previously illustrated in Arabidopsis (Østergaard et al., 2001; Seki et al., 2002)

Finally, several reports indicate that fruit softening is hormonally regulated by ABA and ethylene (Jiang et al., 2000; Zhang et al., 2009a, 2009b). Inhibitors of ABA biosynthesis delay tomato ripening and softening (Zhang et al., 2009a, 2009b). Treatment of berries with ABA or ethephon (an ethylene analog) increases softening (Peppi et al., 2006). In this work, ABA treatment led to modification of the expression of some softening-related genes. Among these, we can cite various xyloglucan endotransglycosylase genes, cellulose synthase genes, and pectinesterase genes. Additionally, genes involved in galactinol (*galactinol synthase*, *stachyose synthase*, and *seed imbibition protein*) and trehalose (*trehalose-6-P phosphatase* and *trehalose-6-P synthase*) metabolism were up-regulated. Both microarray data and the results obtained with transgenic tomatoes strongly indicate the involvement of the ABA-regulated transcription factor VvABF2 in stimulating fruit maturation and softening.

In summary, this work characterizes VvABF2, a transcription factor of the AREB/ABF family sensitive to ABA. This transcription factor mediates at least in part several ABA-mediated pathways controlling both maturation processes and responses to environmental stresses. These include the regulation of secondary metabolism, cell wall metabolism, hormone metabolism and signaling, and stress responses. In addition to its putative role in fruit maturation and because of its general pattern of expression, VvABF2 could be a key component of ABA-mediated grape development and adaptation to environmental cues.

MATERIALS AND METHODS

VvABF2 cDNA Isolation and Production of Constructs for Plant Transformation

A full-length *VvABF2* clone was produced from a cDNA library isolated from grape (*Vitis vinifera* 'Cabernet Sauvignon') berries at the véraison stage. PCR was performed using synthetic oligonucleotide primers designed to begin and end at the start and stop codons of the open reading frame of *VvABF2* (forward primer, 5′-ATGGGGAGTAATTTGAACTTCAAAAACTTC-3′; reverse primer, 5′-CCAGGGGCCAGTCAGTGTGCGTCTCAAGCAA-3′). The complete open reading frame was amplified and cloned into the pGEM-T Easy vector (Promega) for DNA sequencing, prior to subcloning into a stable expression binary vector downstream of the 35S promoter of the *Cauliflower mosaic virus*. pFB8 and Pk7m34GW binary vectors (Gateway; Karimi et al., 2002) were used to generate *VvABF2*-overexpressing 41B cells and tomato (*Solanum lycopersicum*) plants, respectively.

Plant Transformation and Culture Conditions

Grapevine transformations were made in 41B rootstock (cv Chasselas × *Vitis berlandieri*) according to Lecourieux et al. (2010). An embryogenic cell suspension culture was initiated as described previously (Coutos-Thévenot et al., 1992a, 1992b). This cell suspension was subcultured weekly in 25 mL of glycerol-maltose culture medium (Coutos-Thévenot et al., 1992b) supplemented with synthetic auxin (naphthoxyacetic acid) at 1 mg L^{-1} in the dark. Embryogenic cells were transformed using an *Agrobacterium tumefaciens* cocultivation method (Mauro et al., 1995), and after selection, the transgenic cells were subcultured in the same condition in a medium supplemented with paromomycin at 2 mg mL^{-1} and cefotaxime at 200 mg mL^{-1} (Duchefa).

Transgenic tomato plants (cv Wva106) were generated by *A. tumefaciens*-mediated transformation of tomato cotyledons as described by Gonzalez et al. (2007). Tomato plants were grown in a culture chamber with a 14-h-day/10-h-night cycle. The temperature was 25°C during the day and 20°C during the night. Individual flowers were tagged on the day of anthesis (flower opening).

Quantification of Stilbenes

Stilbenes from the culture medium were obtained by a triple ethyl acetate extraction using 5 mL of culture medium. Stilbenoid samples were filtered through 0.45-μm polytetrafluoroethylene membrane filters (Fioroni). Analysis of stilbenes was performed by HPLC on a 250- × 4-mm Prontosil C18 (5 μm) reverse-phase C18 column (Bischoff Chromatography) protected by a guard column of the same material. The HPLC device was coupled to an Esquire 3000 Plus ion-trap mass spectrometer using an electrospray ionization source (Bruker-Daltonics). The chromatographic conditions were not modified, and the HPLC output was split 1:10 in the mass spectrometry detector. Data analysis was performed with Bruker Data Analysis 3.2 software. Separation was performed at a flow rate of 1 mL min^{-1} with a mobile phase composed of water:1% trifluoroacetic acid (97.5:2.5, v/v; A) and acetonitrile:A (80:20, v/v; B). The run was set as follows: 0 to 1 min, 20% B; 1 to 8 min, from 20% to 24% B; 8 to 10 min, from 24% to 25% B; 10 to 13 min, 25% B; 13 to 18 min, from 25% to 30% B; 18 to 35 min, from 30% to 50% B; 35 to 37 min, from 50% to 100% B; 37 to 41 min, 100% B; 41 to 42 min, from 100% to 20% B; and then 20% B for 4 min. UV detection was performed at 286 and 306 nm. Absolute trans-resveratrol and trans-piceid contents were estimated from calibration curves prepared with pure standards. Trans-resveratrol and trans-piceid were purchased from Sigma.

RNA and cDNA Production

Roots, shoots, leaves, and inflorescences were collected from cv Cabernet Sauvignon fruit cuttings grown in a greenhouse. Berries (cv Cabernet Sauvignon) were harvested in Domaine de la Grande Ferrade, Villenave d'Ornon, France. In order to compare berries at the same developmental stage, berries were sorted by weight before véraison and on a NaCl density gradient after véraison.

All collected samples were quickly frozen in liquid nitrogen, ground to a fine powder with a Dangoumau blender, and stored at –80°C prior to use. Total RNA from grape organs and berries was extracted according to Lecourieux et al. (2010). Total RNA from grape cells was extracted from 100 mg of starting tissue using the Spectrum Plant Total RNA Kit (Sigma) following the manufacturer's protocol. RNA isolation was followed by DNaseI treatment. For each sample, RT was performed from 2 μg of purified RNA using Moloney murine leukemia virus reverse transcriptase (Promega) according to the manufacturer's instructions. The cDNA obtained was diluted (1:20) in distilled water.

Gene Expression Analysis

Quantitative real-time RT-PCR expression analysis was carried out using the CFX96 Real-Time PCR Detection system (Bio-Rad). Ten-microliter reaction mixes were prepared, which included 5 μL of iQ SYBR Green Supermix (Bio-Rad), 0.2 μM of each primer, and 2 μL of diluted cDNA. Gene transcripts were

quantified with normalization to *VvEF1γ* (grape experiments [Q9SPF8]; Nicolas et al., 2013) and *SlEiF4α* (tomato experiments [SGN-U578071]) as internal standards. All biological samples were tested in triplicate, and SD values were calculated using standard statistical methods. Specific oligonucleotide primer pairs were designed with Beacon Designer 7 software (Premier Biosoft International). Specific annealing of the oligonucleotides was controlled by dissociation kinetics performed at the end of each PCR run. The efficiency of each primer pair was measured on a PCR product serial dilution. Quantitative real-time RT-PCR primer sequences are listed in Supplemental Table S1.

Protoplast Isolation and Transient Expression Assays

The coding sequence of *VvABF2* was cloned as a C-terminal fusion in frame with the GFP into the pRT101 vector (Kiegerl et al., 2000) and expressed under the control of the cauliflower mosaic virus promoter.

The promoter regions of *VvLEA* (VIT_08s0007g04240), *VvNAC* (VIT_19s0014g03290), and *VvBenzoR* (VIT_07s0005g00140) were inserted upstream of the GUS reporter gene into pAM35 (Guerineau et al., 2003). Primer sequences used for promoter cloning are listed in Supplemental Table S1.

Tobacco (*Nicotiana tabacum* 'SR1') in vitro plants used for protoplast preparation were cultivated in a growth chamber with a constant temperature of 25°C and a 14-h/10-h day/night photoperiod. Protoplasts were obtained from young leaves of 15-d-old subcultured plants according to Hosy et al. (2005). A 10-μg aliquot of each plasmid DNA was used for polyethylene glycol-mediated cotransformation. Transfected protoplasts were incubated for 16 h at 25°C.

GFP fluorescence was analyzed by confocal microscopy. A fluorometric *GUS* assay was performed following the protocol described by Jefferson et al. (1987). Protoplasts were centrifuged briefly for 30 s and vortexed with a GUS extraction buffer containing 50 mM sodium phosphate (pH 7.0), 10 mM β-mercaptoethanol, 10 mM Na_2-EDTA (pH 8.0), and 0.1% Triton X-100. The extracts were centrifuged for 15 min in a microcentrifuge at 4°C, and the supernatants were incubated at 37°C for GUS assay using 1 mM 4-methylumbelliferyl-β-D-glucuronide as a substrate. The reaction was stopped with 0.2 M Na_2CO_3. The amount of methylumbelliferone production was determined using a fluorometer (Versafluor fluorometer; Bio-Rad). Total protein content of the samples was determined by the method of Bradford (1976).

Microarray Experiments and Analyses

Three biological replicates of each sample (untreated control, control + ABA, *35S::VvABF2*, and *35S::VvABF2* + ABA) were hybridized on NimbleGen microarray 090818 Vitis exp HX12 (Roche, NimbleGen), bearing a set of probes for 29,582 unigenes based on the 12X grapevine V1 gene model prediction (http://genomes.cribi.unipd.it/). The chip probe design is available at http://ddlab.sci.univr.it/FunctionalGenomics/. Robust multiarray average-processed data are available at PLEXdb (Wise et al., 2007) with accession number VV30:VvABFOx. Data analyses were performed using R/Bioconductor (Gentleman et al., 2004). Expression intensities were background corrected, quantile normalized, and summarized using the robust multiarray average function of the oligo package (Carvalho and Irizarry, 2010). Differentially expressed genes were identified using the Limma package (Smyth, 2004) for the following contrasts: *35S::VvABF2* versus control, *35S::VvABF2* + ABA versus control, and control + ABA versus control. Genes with absolute $\log_2$-fold changes above 1 (ratio of 2) and false discovery rate (Benjamini and Hochberg, 1995)-corrected *P* values below 0.05 were considered significant. Expression profiles of these genes were clustered using the MapMan software (Thimm et al., 2004). Gene models were aligned against the UnirRef100-2011-07 database (Suzek et al., 2007) using the BLAST program (Altschul et al., 1997). Genes considered as differentially expressed were associated with the MapMan Ontology (Thimm et al., 2004) using the Mercator Online tool (http://mapman.gabipd.org/web/guest/app/mercator). The most extreme categories within these differentially expressed genes were identified using a Wilcoxon rank-sum test implemented in the MapMan tool (Usadel et al., 2005).

Sequence Analysis

Full-length amino acid sequences of bZIP transcription factors from several species were retrieved from public databases. Multiple amino acid sequence alignment was generated using the Clustal Omega alignment algorithm with default parameters (Sievers et al., 2011; http://www.ebi.ac.uk/Tools/msa/clustalo/). The phylogenetic tree was constructed from the Clustal Omega alignment using the MEGA5 package (Tamura et al., 2011). The evolutionary history was inferred using the neighbor-joining method. The optimal tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and were in units of the number of amino acid substitutions per site. All positions containing gaps and missing data were eliminated from the data set (complete deletion option). There were a total of 117 positions in the final data set.

In Silico *VvABF2* Promoter Region Identification and Analysis

The identification and in silico analysis of the *VvABF2* promoter region were conducted using the Genomatix suite of programs (http://www.genomatix.de [Genomatix Software]; Quandt et al., 1995). The Gene2promotor program from the Genomatix software package was used to define 1,344 bp upstream of the transcription start site of the *VvABF2* promoter region. The corresponding sequence was then used as the target sequence for transcription factor recognition site identification using the MatInspector version 8.06 program (Cartharius et al., 2005). The parameters used were the Matrix Family Library version 8.4 (June 2011), the standard (0.75) core similarity, and the optimized matrix similarity.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. VvABF2 binds to promoter fragments containing ABRE motifs.

Supplemental Figure S2. Time course of total stilbene production (nmol g^{-1} dry weight) in the culture medium of control and 35S::*VvABF2* transgenic cells after the addition of 20 μM ABA.

Supplemental Figure S3. Mature fruit phenotypes of *VvABF2*-overexpressing tomato plants.

Supplemental Table S1. Potential ABRE cis-acting elements identified in the 5′ regulatory region of *VvLEA*, *VvBenzoR*, and *VvNAC*.

Supplemental Table S2. Potential cis-acting elements identified in the 5′ regulatory region of *VvABF2*.

Supplemental Table S3. Genes involved in the response to oxidative stress and affected in the response to ABA and/or *VvABF2* overexpression.

Supplemental Table S4. PCR primers used to amplify gene- and promoter-specific regions for cloning and expression analyses.

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LITERATURE CITED

Adams-Phillips L, Barry C, Giovannoni J (2004) Signal transduction systems regulating fruit ripening. Trends Plant Sci **9:** 331–338

Aharoni A, O'Connell AP (2002) Gene expression analysis of strawberry achene and receptacle maturation using DNA microarrays. J Exp Bot **53:** 2073–2087

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res **25:** 3389–3402

Amir Hossain M, Lee Y, Cho JI, Ahn CH, Lee SK, Jeon JS, Kang H, Lee CH, An G, Park PB (2010) The bZIP transcription factor OsABF1 is an ABA responsive element binding factor that enhances abiotic stress signaling in rice. Plant Mol Biol **72:** 557–566

Antolín MC, Baigorri H, De Luis I, Aguirrezábal F, Geny L, Broquedis M, Sánchez-Díaz M (2003) ABA during reproductive development in

non-irrigated grapevines (Vitis vinifera L. cv. Tempranillo). Aust J Grape Wine Res **9:** 169–176

Antoni R, Rodriguez L, Gonzalez-Guzman M, Pizzio GA, Rodriguez PL (2011) News on ABA transport, protein degradation, and ABFs/WRKYs in ABA signaling. Curr Opin Plant Biol **14:** 547–553

Ban T, Ishimaru M, Kobayashi S, Goto-Yamamoto SN, Horiuchi S (2003) Abscisic acid and 2,4-dichlorophenoxyacetic acid affect the expression of anthocyanin biosynthetic pathway genes in 'Kyoho' grape berries. J Hortic Sci Biotechnol **78:** 586–589

Bastías A, López-Climent M, Valcárcel M, Rosello S, Gómez-Cadenas A, Casaretto JA (2011) Modulation of organic acids and sugar content in tomato fruits by an abscisic acid-regulated transcription factor. Physiol Plant **141:** 215–226

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc **57:** 289–300

Böttcher C, Boss PK, Davies C (2011) Acyl substrate preferences of an IAA-amido synthetase account for variations in grape (*Vitis vinifera* L.) berry ripening caused by different auxinic compounds indicating the importance of auxin conjugation in plant development. J Exp Bot **62:** 4267–4280

Böttcher C, Keyzers RA, Boss PK, Davies C (2010) Sequestration of auxin by the indole-3-acetic acid-amido synthetase GH3-1 in grape berry (Vitis vinifera L.) and the proposed role of auxin conjugation during ripening. J Exp Bot **61:** 3615–3625

Bradamante S, Barenghi L, Villa A (2004) Cardiovascular protective effects of resveratrol. Cardiovasc Drug Rev **22:** 169–188

Buchanan CD, Lim S, Salzman RA, Kagiampakis I, Morishige DT, Weers BD, Klein RR, Pratt LH, Cordonnier-Pratt MM, Klein PE, et al (2005) *Sorghum bicolor*'s transcriptome response to dehydration, high salinity and ABA. Plant Mol Biol **58:** 699–720

Cartharius K, Frech K, Grote K, Klocke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M, Werner T (2005) MatInspector and beyond: promoter analysis based on transcription factor binding sites. Bioinformatics **21:** 2933–2942

Carvalho BS, Irizarry RA (2010) A framework for oligonucleotide microarray preprocessing. Bioinformatics **26:** 2363–2367

Chai YM, Jia HF, Li CL, Dong QH, Shen YY (2011) FaPYR1 is involved in strawberry fruit ripening. J Exp Bot **62:** 5079–5089

Chervin C, El-Kereamy A, Roustan JP, Latche A, Lamon J, Bouzayen M (2004) Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. Plant Sci **167:** 1301–1305

Chervin C, Tira-Umphon A, Terrier N, Zouine M, Severac D, Roustan JP (2008) Stimulation of the grape berry expansion by ethylene and effects on related gene transcripts, over the ripening phase. Physiol Plant **134:** 534–546

Choi H, Hong J, Ha J, Kang J, Kim SY (2000) ABFs, a family of ABA-responsive element binding factors. J Biol Chem **275:** 1723–1730

Coombe BG (1992) Research on development and ripening of the grape berry. Am J Enol Vitic **43:** 101–110

Coombe BG, Hale CR (1973) The hormone content of ripening grape berries and the effects of growth substance treatments. Plant Physiol **51:** 629–634

Corrêa LG, Riaño-Pachón DM, Schrago CG, dos Santos RV, Mueller-Roeber B, Vincentz M (2008) The role of bZIP transcription factors in green plant evolution: adaptive features emerging from four founder genes. PLoS ONE **3:** e2944

Coutos-Thévenot P, Goebel-Tourand I, Mauro MC, Jouanneau JP, Boulay M, Deloire A, Guern J (1992a) Somatic embryogenesis from grapevine cells. I. Improvement of embryo development by changes in culture conditions. Plant Cell Tissue Organ Cult **29:** 125–133

Coutos-Thévenot P, Maës O, Jouenne T, Mauro MC, Boulay M, Deloire A, Guern J (1992b) Extracellular protein patterns of grapevine cell suspensions in embryogenic and non-embryogenic situations. Plant Sci **86:** 137–145

Cuéllar T, Azeem F, Andrianteranagna M, Pascaud F, Verdeil JL, Sentenac H, Zimmermann S, Gaillard I (2013) Potassium transport in developing fleshy fruits: the grapevine inward K(+) channel VvK1.2 is activated by CIPK-CBL complexes and induced in ripening berry flesh cells. Plant J **73:** 1006–1018

Cuéllar T, Pascaud F, Verdeil JL, Torregrosa L, Adam-Blondon AF, Thibaud JB, Sentenac H, Gaillard I (2010) A grapevine Shaker inward K(+) channel activated by the calcineurin B-like calcium sensor 1-protein kinase CIPK23 network is expressed in grape berries under drought stress conditions. Plant J **61:** 58–69

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol **61:** 651–679

Davies C, Boss PK, Robinson SP (1997) Treatment of grape berries, a nonclimacteric fruit with a synthetic auxin, retards ripening and alters the expression of developmentally regulated genes. Plant Physiol **115:** 1155–1161

Davies C, Robinson SP (2000) Differential screening indicates a dramatic change in mRNA profiles during grape berry ripening: cloning and characterization of cDNAs encoding putative cell wall and stress response proteins. Plant Physiol **122:** 803–812

Davies C, Shin R, Liu W, Thomas MR, Schachtman DP (2006) Transporters expressed during grape berry (*Vitis vinifera* L.) development are associated with an increase in berry size and berry potassium accumulation. J Exp Bot **57:** 3209–3216

Deluc LG, Quilici DR, Decendit A, Grimplet J, Wheatley MD, Schlauch KA, Mérillon JM, Cushman JC, Cramer GR (2009) Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. BMC Genomics **10:** 212

Desikan R, A-H-Mackerness S, Hancock JT, Neill SJ (2001) Regulation of the Arabidopsis transcriptome by oxidative stress. Plant Physiol **127:** 159–172

Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park SY, Cutler SR, Sheen J, Rodriguez PL, Zhu JK (2009) In vitro reconstitution of an abscisic acid signalling pathway. Nature **462:** 660–664

Furihata T, Maruyama K, Fujita Y, Umezawa T, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2006) Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. Proc Natl Acad Sci USA **103:** 1988–1993

Gagné S, Cluzet S, Merillon JM, Gény L (2011) ABA initiates anthocyanin production in grape cell cultures. J Plant Growth Regul **30:** 1–10

Gambetta GA, Matthews MA, Shaghasi TH, McElrone AJ, Castellarin SD (2010) Sugar and abscisic acid signaling orthologs are activated at the onset of ripening in grape. Planta **232:** 219–234

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, et al (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol **5:** R80

Guerineau F, Benjdia M, Zhou DX (2003) A jasmonate-responsive element within the A. thaliana vsp1 promoter. J Exp Bot **54:** 1153–1162

Giovannoni J (2001) Molecular biology of fruit maturation and ripening. Annu Rev Plant Physiol Plant Mol Biol **52:** 725–749

Giovannoni JJ (2004) Genetic regulation of fruit development and ripening. Plant Cell (Suppl) **16:** S170–S180

Giovannoni JJ (2007) Fruit ripening mutants yield insights into ripening control. Curr Opin Plant Biol **10:** 283–289

Giribaldi M, Gény L, Delrot S, Schubert A (2010) Proteomic analysis of the effects of ABA treatments on ripening *Vitis vinifera* berries. J Exp Bot **61:** 2447–2458

Gomez C, Terrier N, Torregrosa L, Vialet S, Fournier-Level A, Verriès C, Souquet JM, Mazauric JP, Klein M, Cheynier V, et al (2009) Grapevine MATE-type proteins act as vacuolar H^+-dependent acylated anthocyanin transporters. Plant Physiol **150:** 402–415

Gómez-Porras JL, Riaño-Pachón DM, Dreyer I, Mayer JE, Mueller-Roeber B (2007) Genome-wide analysis of ABA-responsive elements ABRE and CE3 reveals divergent patterns in Arabidopsis and rice. BMC Genomics **8:** 260–273

Gonzalez N, Gévaudant F, Hernould M, Chevalier C, Mouras A (2007) The cell cycle-associated protein kinase WEE1 regulates cell size in relation to endoreduplication in developing tomato fruit. Plant J **51:** 642–655

Hardtke CS (2007) Transcriptional auxin-brassinosteroid crosstalk: who's talking? Bioessays **29:** 1115–1123

Hattori T, Totsuka M, Hobo T, Kagaya Y, Yamamoto-Toyoda A (2002) Experimentally determined sequence requirement of ACGT-containing abscisic acid response element. Plant Cell Physiol **43:** 136–140

Hofseth LJ, Singh UP, Singh NP, Nagarkatti M, Nagarkatti PS (2010) Taming the beast within: resveratrol suppresses colitis and prevents colon cancer. Aging (Albany NY) **2:** 183–184

Hosy E, Duby G, Véry AA, Costa A, Sentenac H, Thibaud JB (2005) A procedure for localisation and electrophysiological characterisation of

ion channels heterologously expressed in a plant context. Plant Methods **1:** 14

Jakoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in Arabidopsis. Trends Plant Sci **7:** 106–111

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J **6:** 3901–3907

Jeong ST, Goto-Yamamoto N, Kobayashi S, Esaka M (2004) Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. Plant Sci **167:** 247–252

Jia HF, Chai YM, Li CL, Lu D, Luo JJ, Qin L, Shen YY (2011) Abscisic acid plays an important role in the regulation of strawberry fruit ripening. Plant Physiol **157:** 188–199

Jiang Y, Deyholos MK (2009) Functional characterization of Arabidopsis NaCl-inducible WRKY25 and WRKY33 transcription factors in abiotic stresses. Plant Mol Biol **69:** 91–105

Jiang Y, Joyce DC, Macnish AJ (2000) Effect of abscisic acid on banana fruit ripening in relation to the role of ethylene. J Plant Growth Regul **19:** 106–111

Jiménez A, Creissen G, Kular B, Firmin J, Robinson S, Verhoeyen M, Mullineaux P (2002) Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. Planta **214:** 751–758

Kang JY, Choi HI, Im MY, Kim SY (2002) *Arabidopsis* basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. Plant Cell **14:** 343–357

Karimi M, Inzé D, Depicker A (2002) Gateway vectors for *Agrobacterium*-mediated plant transformation. Trends Plant Sci **7:** 193–195

Kiegerl S, Cardinale F, Siligan C, Gross A, Baudouin E, Liwosz A, Eklöf S, Till S, Bögre L, Hirt H, et al (2000) SIMKK, a mitogen-activated protein kinase (MAPK) kinase, is a specific activator of the salt stress-induced MAPK, SIMK. Plant Cell **12:** 2247–2258

Kobashi K, Gemma H, Iwahori S (1999) Sugar accumulation in peach fruit as affected by abscisic acid treatment in relation to some sugar metabolizing enzymes. J Jpn Soc Hortic Sci **68:** 465–470

Koyama K, Sadamatsu K, Goto-Yamamoto N (2010) Abscisic acid stimulated ripening and gene expression in berry skins of the Cabernet Sauvignon grape. Funct Integr Genomics **10:** 367–381

Lecourieux F, Lecourieux D, Vignault C, Delrot S (2010) A sugar-inducible protein kinase, VvSK1, regulates hexose transport and sugar accumulation in grapevine cells. Plant Physiol **152:** 1096–1106

Li G, Xin H, Zheng XF, Li S, Hu Z (2012) Identification of the abscisic acid receptor VvPYL1 in *Vitis vinifera*. Plant Biol (Stuttg) **14:** 244–248

Li X, Xu CJ, Korban SS, Chen KS (2010) Regulatory mechanisms of textural changes in ripening fruits. Crit Rev Plant Sci **29:** 222–243

Liu H, Stone SL (2010) Abscisic acid increases *Arabidopsis* ABI5 transcription factor levels by promoting KEG E3 ligase self-ubiquitination and proteasomal degradation. Plant Cell **22:** 2630–2641

Liu X, Yue Y, Li B, Nie Y, Li W, Wu WH, Ma L (2007) A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid. Science **315:** 1712–1716

Matsui A, Ishida J, Morosawa T, Mochizuki Y, Kaminuma E, Endo TA, Okamoto M, Nambara E, Nakajima M, Kawashima M, et al (2008) *Arabidopsis* transcriptome analysis under drought, cold, high-salinity and ABA treatment conditions using a tiling array. Plant Cell Physiol **49:** 1135–1149

Mauro MC, Toutain S, Walter B, Pinck L, Otten L, Coutos-Thévenot P, Deloire A, Barbier P (1995) High efficiency regeneration of grapevine plants transformed with the GFLV coat protein gene. Plant Sci **112:** 97–106

Melcher K, Ng LM, Zhou XE, Soon FF, Xu Y, Suino-Powell KM, Park SY, Weiner JJ, Fujii H, Chinnusamy V, et al (2009) A gate-latch-lock mechanism for hormone signalling by abscisic acid receptors. Nature **462:** 602–608

Miyazono K, Miyakawa T, Sawano Y, Kubota K, Kang H-J, Asano A, Miyauchi Y, Takahashi M, Zhi Y, Fujita Y, et al (2009) Structural basis of abscisic acid signalling. Nature **462:** 609–614

Mok MC (1994) Cytokinins and plant development: an overview. *In* DWS Mok, MC Mok, eds, Cytokinins: Chemistry, Activity and Function. CRC Press, Boca Raton, FL, pp 155–156

Nakashima K, Fujita Y, Kanamori N, Katagiri T, Umezawa T, Kidokoro S, Maruyama K, Yoshida T, Ishiyama K, Kobayashi M, et al (2009) Three Arabidopsis SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, involved in ABA signaling are essential for the control of seed development and dormancy. Plant Cell Physiol **50:** 1345–1363

Nemhauser JL, Hong F, Chory J (2006) Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell **126:** 467–475

Nicolas P, Lecourieux D, Gomès E, Delrot S, Lecourieux F (2013) The grape berry-specific basic helix-loop-helix transcription factor VvCEB1 affects cell size. J Exp Bot **64:** 991–1003

Nishimura N, Hitomi K, Arvai AS, Rambo RP, Hitomi C, Cutler SR, Schroeder JI, Getzoff ED (2009) Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. Science **326:** 1373–1379

Nishimura N, Yoshida T, Kitahata N, Asami T, Shinozaki K, Hirayama T (2007) ABA-Hypersensitive Germination1 encodes a protein phosphatase 2C, an essential component of abscisic acid signaling in Arabidopsis seed. Plant J **50:** 935–949

Østergaard L, Lauvergeat V, Naested H, Mattsson O, Mundy J (2001) Two differentially regulated Arabidopsis genes define a new branch of the DFR superfamily. Plant Sci **160:** 463–472

Pan QH, Li MJ, Peng CC, Zhang N, Zou X, Zou KQ, Wang XL, Yu XC, Wang XF, Zhang DP (2005) Abscisic acid activates acid invertases in developing grape berry. Physiol Plant **125:** 157–170

Pandey S, Nelson DC, Assmann SM (2009) Two novel GPCR-type G proteins are abscisic acid receptors in Arabidopsis. Cell **136:** 136–148

Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF, et al (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science **324:** 1068–1071

Peppi MC, Fidelibus MW, Dokoozlian N (2006) Abscisic acid application timing and concentration affect firmness, pigmentation, and color of 'Flame Seedless' grapes. Hortic Sci **41:** 1440–1445

Pezet R, Gindro K, Viret O, Spring JL (2004) Glycosylation and oxidative dimerization of resveratrol are respectively associated to sensitivity and resistance of grapevine cultivars to downy mildew. Physiol Mol Plant Pathol **65:** 297–303

Pilati S, Perazzolli M, Malossini A, Cestaro A, Demattè L, Fontana P, Dal Ri A, Viola R, Velasco R, Moser C (2007) Genome-wide transcriptional analysis of grapevine berry ripening reveals a set of genes similarly modulated during three seasons and the occurrence of an oxidative burst at vèraison. BMC Genomics **8:** 428

Quandt K, Frech K, Karas H, Wingender E, Werner T (1995) MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. Nucleic Acids Res **23:** 4878–4884

Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, Yoshiwara K, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. Plant Physiol **133:** 1755–1767

Ribéreau-Gayon Y, Glories Y, Maujean A, Dubourdieu D (2000) Handbook of Enology, Vol 2: The Chemistry of Wine and Stabilization and Treatments. John Wiley & Sons, Chichester, England

Richings EW, Cripps RF, Cowan AK (2000) Factors affecting 'Hass' avocado fruit size: carbohydrate, abscisic acid and soprenoid metabolism in normal and phenotypically small fruit. Physiol Plant **109:** 81–89

Rodrigo MJ, Marcos JF, Alférez F, Mallent MD, Zacarías L (2003) Characterization of Pinalate, a novel Citrus sinensis mutant with a fruit-specific alteration that results in yellow pigmentation and decreased ABA content. J Exp Bot **54:** 727–738

Santiago J, Dupeux F, Round A, Antoni R, Park SY, Jamin M, Cutler SR, Rodriguez PL, Márquez JA (2009) The abscisic acid receptor PYR1 in complex with abscisic acid. Nature **462:** 665–668

Scienza A, Miravalle CV, Fregoni M (1978) Relationships between seed number, gibberellin and abscisic acid levels and ripening in 'Cabernet Sauvignon' grape berries. Vitis **17:** 361–368

Seki M, Ishida J, Narusaka M, Fujita M, Nanjo T, Umezawa T, Kamiya A, Nakajima M, Enju A, Sakurai T, et al (2002) Monitoring the expression pattern of around 7,000 Arabidopsis genes under ABA treatments using a full-length cDNA microarray. Funct Integr Genomics **2:** 282–291

Seo PJ, Xiang F, Qiao M, Park JY, Lee YN, Kim S-G, Lee YH, Park WJ, Park CM (2009) The MYB96 transcription factor mediates abscisic acid signaling during drought stress response in Arabidopsis. Plant Physiol **151:** 275–289

Seymour GB, Taylor JE, Tucker GA (1993) Biochemistry of Fruit Ripening. Chapman and Hall, London

Shang Y, Yan L, Liu ZQ, Cao Z, Mei C, Xin Q, Wu FQ, Wang XF, Du SY, Jiang T, et al (2010) The Mg-chelatase H subunit of *Arabidopsis* antagonizes a group of WRKY transcription repressors to relieve ABA-responsive genes of inhibition. Plant Cell **22:** 1909–1935

Shen YY, Wang XF, Wu FQ, Du SY, Cao Z, Shang Y, Wang XL, Peng CC, Yu XC, Zhu SY, et al (2006) The Mg-chelatase H subunit is an abscisic acid receptor. Nature **443:** 823–826

Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, et al (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol **7:** 539

Smalle J, Vierstra RD (2004) The ubiquitin 26S proteasome proteolytic pathway. Annu Rev Plant Biol **55:** 555–590

Smyth GK (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol **3:** e3

Sun L, Wang YP, Chen P, Ren J, Ji K, Li Q, Li P, Dai SJ, Leng P (2011) Transcriptional regulation of *SlPYL, SlPP2C,* and *SlSnRK2* gene families encoding ABA signal core components during tomato fruit development and drought stress. J Exp Bot **62:** 5659–5669

Suzek BE, Huang H, McGarvey P, Mazumder R, Wu CH (2007) UniRef: comprehensive and non-redundant UniProt reference clusters. Bioinformatics **23:** 1282–1288

Symons GM, Davies C, Shavrukov Y, Dry IB, Reid JB, Thomas MR (2006) Grapes on steroids: brassinosteroids are involved in grape berry ripening. Plant Physiol **140:** 150–158

Szkudelska K, Szkudelski T (2010) Resveratrol, obesity and diabetes. Eur J Pharmacol **635:** 1–8

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol **28:** 2731–2739

Thimm O, Bläsing O, Gibon Y, Nagel A, Meyer S, Krüger P, Selbig J, Müller LA, Rhee SY, Stitt M (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J **37:** 914–939

Tosti N, Pasqualini S, Borgogni A, Ederli L, Falistocco E, Crispi S, Paolocci F (2006) Gene expression profiles of O3-treated Arabidopsis plants. Plant Cell Environ **29:** 1686–1702

Umezawa T, Sugiyama N, Mizoguchi M, Hayashi S, Myouga F, Yamaguchi-Shinozaki K, Ishihama Y, Hirayama T, Shinozaki K (2009) Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in Arabidopsis. Proc Natl Acad Sci USA **106:** 17588–17593

Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000) Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. Proc Natl Acad Sci USA **97:** 11632–11637

Usadel B, Nagel A, Thimm O, Redestig H, Blaesing OE, Palacios-Rojas N, Selbig J, Hannemann J, Piques MC, Steinhauser D, et al (2005) Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of corresponding genes, and comparison with known responses. Plant Physiol **138:** 1195–1204

Vanderauwera S, De Block M, Van de Steene N, van de Cotte B, Metzlaff M, Van Breusegem F (2007) Silencing of poly(ADP-ribose) polymerase in plants alters abiotic stress signal transduction. Proc Natl Acad Sci USA **104:** 15150–15155

Wang RS, Pandey S, Li S, Gookin TE, Zhao Z, Albert R, Assmann SM (2011) Common and unique elements of the ABA-regulated transcriptome of Arabidopsis guard cells. BMC Genomics **12:** 216

Weiner JJ, Peterson FC, Volkman BF, Cutler SR (2010) Structural and functional insights into core ABA signaling. Curr Opin Plant Biol **13:** 495–502

Wheeler S, Loveys B, Ford C, Davies C (2009) The relationship between the expression of abscisic acid biosynthesis genes, accumulation of abscisic acid and the promotion of Vitis vinifera L. berry ripening by abscisic acid. Aust J Grape Wine Res **15:** 195–204

Wise RP, Caldo RA, Hong L, Shen L, Cannon EK, Dickerson JA (2007) BarleyBase/PLEXdb: a unified expression profiling database for plants and plant pathogens. Methods Mol Biol **406:** 347–363

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu Rev Plant Biol **57:** 781–803

Yamaki S, Asakura T (1991) Stimulation of the uptake of sorbitol into vacuoles from apple fruit flesh by abscisic acid and into protoplasts by indoleacetic acid. Plant Cell Physiol **32:** 315–318

Yoshida T, Fujita Y, Sayama H, Kidokoro S, Maruyama K, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2010) AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. Plant J **61:** 672–685

Yu XC, Li MJ, Gao GF, Feng HZ, Geng XQ, Peng CC, Zhu SY, Wang XJ, Shen YY, Zhang DP (2006) Abscisic acid stimulates a calcium-dependent protein kinase in grape berry. Plant Physiol **140:** 558–579

Zhang M, Leng P, Zhang G, Li X (2009a) Cloning and functional analysis of 9-cis-epoxycarotenoid dioxygenase (NCED) genes encoding a key enzyme during abscisic acid biosynthesis from peach and grape fruits. J Plant Physiol **166:** 1241–1252

Zhang M, Yuan B, Leng P (2009b) The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. J Exp Bot **60:** 1579–1588

Zhang S, Cai Z, Wang X (2009) The primary signaling outputs of brassinosteroids are regulated by abscisic acid signaling. Proc Natl Acad Sci USA **106:** 4543–4548

Zhao J, Dixon RA (2009) MATE transporters facilitate vacuolar uptake of epicatechin 3′-O-glucoside for proanthocyanidin biosynthesis in *Medicago truncatula* and *Arabidopsis*. Plant Cell **21:** 2323–2340