Abscisic acid stimulated ripening and gene expression in berry skins of the Cabernet Sauvignon grape

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Abstract We investigated the effect of exogenous abscisic acid (ABA) application on the transcriptome as well as the phenolic profiles in the skins of *Vitis vinifera* cv. Cabernet Sauvignon grape berries grown on the vine and cultured in vitro. ABA application rapidly induced the accumulation of anthocyanin and flavonol. Correlatively, the structural genes in the phenylpropanoid and flavonoid pathways, their transcriptional regulators, as well as genes considered to be involved in the acylation and transport of anthocyanin into the vacuole, were upregulated by ABA treatment. The Genechip analysis showed that the ABA treatment significantly up- or downregulated a total of 345 and 1,482 transcripts in the skins of berries grown on the vine and cultured in vitro, respectively. Exogenous ABA modulated the transcripts associated with osmotic responses, stress responses, cell wall modification, auxin and ethylene metabolism and responses, in addition to the induction of anthocyanin biosynthetic genes, and reduced those associated with photosynthesis; approximately half of these transcripts were identical to the previously reported ripening-specific genes.

Keywords Abscisic acid (ABA) · Ripening · Anthocyanin biosynthesis · Affymetrix oligonucleotide array · Berry skins · *Vitis vinifera*

Introduction

Grapes are cultivated worldwide, and the berries and their derivatives, in particular, wine, are economically important. The grape is a nonclimacteric fruit, and berry development consists of two successive sigmoidal growth periods separated by a lag phase (Coombe 1976). The period of transition is called veraison, when the metabolism in berries changes markedly toward ripening (Deluc et al. 2007).

The grape molecular biology which aims to understand the grape berry development and ripening has developed unprecedentedly. The powerful resources, such as whole genome sequence of *Vitis vinifera* Pinot Noir (Jaillon et al. 2007; Velasco et al. 2007) and the most extensive expressed sequence tags (ESTs) collection amongst fruit species with more than 23,000 unigenes in National Center Biotechnology Information database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene), are now available, making grape as a model plant for studies on non-climacteric anthocyanin accumulating fruits. Several large-scale and non-targeted systematic approaches, such as differential screening and DNA microarray analysis, were utilized to understand, in particular, the physiology during berry development and ripening (Davies and Robinson 2000; Terrier et al. 2005; Waters et al. 2005; Grimplet et al. 2007; Pilati et al. 2007; Deluc et al. 2007). A core set of 1,477 genes specifically involved in grape berry ripening in three growing seasons was extracted by means of Affymetrix *Vitis* Genechip (Pilati et al. 2007). Five functional categories: cell wall organization and biogenesis, carbohydrate- and secondary metabolisms, stress response, photosynthesis, were overrepresented in this set. However, the understanding of the transcriptional regulatory hierarchies of these ripening regulated genes is still lacking. Although many exogenous and endogenous signals, such as abscisic acid (ABA), brassinosteroids, ethylene, and hexoses, have been implicated in control of the ripening of the berries, but the mechanisms acting at the molecular levels require further clarification (Inaba et al. 1974; Çakir et al. 2003; Chervin et al. 2004; Lund et al. 2008).

ABA is believed to play an important role for the regulation of berry ripening, as the endogenous ABA concentrations in the berries increase dramatically at the onset of ripening (Coombe and Hale

1973; Düring et al. 1978). Exogenous ABA application on pre-veraison berries hastened berry ripening (Inaba et al. 1974).

ABA also plays a key role in plant adaptation to adverse environmental conditions, including drought. ABA accumulation controls the various downstream responses for the plant to acquire tolerance to these stresses (Seki et al. 2002; Rabani et al. 2003; Shinozaki and Yamaguchi-Shinozaki 2007; Huang et al. 2008). The various biochemical and physiological responses induced by exogenous ABA application were studied at the transcriptional levels by using genome-wide DNA microarray and tiling array (Seki et al. 2002; Huang et al. 2007; Matsui et al. 2008). For example, 245 ABA-inducible genes were identified in the *Arabidopsis* plantlet (Seki et al. 2002). These included the proteins that function in stress tolerance, such as late-embryogenesis-abundant (LEA) proteins, heat-shock proteins, osmoprotectant-biosynthesis-related proteins, detoxification enzymes, and senescence-related proteins as well as the regulatory proteins.

Consistent with the role of ABA as a stress-inducible hormone, endogenous ABA concentrations in the grape berries during ripening were influenced by stress treatment such as water-deficit irrigation (Okamoto et al. 2004; Deluc et al. 2009). The modification of the ABA levels by these treatments was correlated to the progress of ripening, particularly, to coloring in the berry skins. Exogenous ABA applications on the berries were reported to improve the berry coloring of both table grapes and wine grapes (Yakushiji et al. 2001; Jeong et al. 2004; Mori et al. 2005; Peppi et al. 2007; Giribaldi et al. 2009; Wheeler et al. 2009). Differently from the water-deficit treatment, the ABA applications had few effects on the fruit size and composition, such as the sugar and acid concentrations. The increased skin fresh weight, as well as the increased coloration, was speculated to be the result of advanced skin maturation by the treatment (Peppi et al. 2008). However, the specific role of ABA in the berry skins during ripening is still not clear.

The objective of this study was to comprehensively examine the effect of exogenous ABA application on the transcriptome as well as the phenolic profiles in the berry skins of Cabernet Sauvignon grape.

Materials and methods

Plant material and treatments

The field experiment was conducted in 2007 using grapevines of Cabernet Sauvignon grown in an experimental vineyard in Higashi-Hiroshima, Japan. Vines were trained on a Guyot trellising system (Jackson 2000), and each vine carried 10-20 bunches of grapes. Vines were divided into three sets to provide three biological replicates. At veraison, half of the clusters of each vine were sprayed with a 400 mg l^{-1} abscisic acid ((+)-ABA) solution prepared by the method of Mori et al. (2005). Eighty berries were sampled randomly every two weeks until harvest (37 days after veraison (DAV)) from each treatment plot. Berry skins were peeled manually and immediately frozen in liquid nitrogen and stored at -80°C until use.

Grapes of each treatment were harvested, and an 800-g lot of berries were used for small-scale vinification trial according to the method of Koyama et al. (2007). The fermentation trials were triplicated in a thermostatic chamber (25°C) during 8 days.

At veraison, softened green berries of the same field-grown Cabernet Sauvignon vines used for the field treatments were excised from the rachis and cultured on the media with and without 750 mg l^{-1} (±)-ABA (Sigma-Aldrich, Inc., St. Louis, MO, USA), according to the method described previously (Mori et al. 2007). Three and 10 days after the treatment, the berry skins were sampled from each treatment as stated above. The experiments were triplicated.

Phenolic concentration and composition

Skin phenolics were extracted from the berry skins with the method of Mané et al. (2007). The dried extracts were dissolved in 80% methanol and used for the high-performance liquid chromatography (HPLC) analysis of monomeric phenolics, and the extracts were also subjected to the phloroglucinolysis of proanthocyanidins, as described previously (Koyama et al. 2007).

RNA isolation and Genechip analysis

Total RNA was extracted and purified from one gram of pooled berry skin according to the method of Reid et al. (2006). The quality of the total RNA was examined with the RNA 6000 Nano Assay on the Agilent 2100 Bioanalyzer (Agilent Technologies, Wilmington, DE, USA) according to the manufacturer's instructions. Five μg of total RNA was used for synthesis of cRNA, hybridization, and scanning, according to Affymetrix-recommended protocol. The image data of three biological replicates for all samples were collected and processed using Genechip Operating Software (GCOS 1.2). A global scaling factor of 500, a normalization value of 1, and a default parameter setting for the *Vitis vinifera* Genechip were used. The probe values in an array were adjusted with 75-percentile shift normalization. First, probe sets that had been flagged "present" at all of the biological replicates of at least one condition were retained for further analysis. Second, the subset of probe sets was further extracted on the basis of 2-fold changes as compared with the control at each sampling time. Third, to determine the probe sets whose abundance differed significantly between the ABA treatments and the control, a *t*-test assuming the equal variance (p values <0.05) was further performed. A multiple testing correction (Benjamini and Hochberg 1995) was performed on the *t* statistics of each contrast to adjust the false discovery rate. Clustering was carried out on the basis of a relative expression pattern under ABA treatment during the ripening period by the k-means method (k=6) with Pearson's correlation distance using the T-MeV software (http://www.tm4.org). The annotations and the functional categories of these probe sets were acquired through the updated Genechip master list developed by Deluc et al. (2007). The functional category distribution in the differentially expressed transcripts under ABA treatment and that in the entire chip sequences were compared by means of the Chi square test (p value<0.05).

ABA analysis

Free (±)-ABA concentration in the skin sample of each vine was determined by gas chromatograph-mass spectrometry according to the method of Koshita et al. (1999). The

concentration of ABA in the original extract was determined from the ratio of the peak areas of m/z 190 ($^2H_0$) to those of m/z 194 ($^2H_6$) analyzed by gas chromatography equipped with a mass spectrometer (Polaris Q, Thermo Fisher Scientific, Waltham, MA, USA) and expressed per g fresh weight (FW).

Results and discussion

Effects of ABA treatments on berry composition and phenolic profile in the skins of berries grown on the vine and cultured in vitro

ABA concentrations in the skins of ABA-sprayed berries grown on the vine were about 14-fold higher than those of the control even at 14 days after the treatment (0.37 $\mu g \cdot g^{-1}$ skin and 5.28 $\mu g \cdot g^{-1}$ skin in the control and ABA-treated berries, respectively, at 14 DAV; 0.31 $\mu g \cdot g^{-1}$ skin and 3.31 $\mu g \cdot g^{-1}$ in the control and ABA-treated berries, respectively, at 28 DAV), indicating that ABA continuously stimulated the grape berries for a long time.

The soluble solid concentrations were slightly higher in the ABA-treated berries than in the control at 14 and 28 DAV, while no differences were observed at harvest (Supplemental Table S1). No differences in the berry weight, titratable acidity, and pH were observed at any sampling time. Remarkable differences were observed on the phenolic profiles in the berry skins (Table 1). As expected, the anthocyanin concentrations in ABA-treated berries were more than 2-fold higher than those in the control berries during ripening. The anthocyanin concentrations in the ABA-treated berries were already maximized at 14 DAV and remained relatively stable throughout ripening, while the maximum concentrations in the control berries were observed at harvest. In addition, the modification of the anthocyanin composition was significant in the ABA-treated berries throughout ripening. In detail, the proportion of acylated (acetyl- and *p*-coumaryl-) anthocyanins were lower, and that of less methoxylated- anthocyanins (delphinidin, cyanidin, and petunidin) relative to peonidin and malvidin were higher than that of the control berries. Throughout ripening, the flavonol concentrations were higher in the ABA-treated berry skins than those in the control berries. There

were no differences in the cinnamic acid concentration or in the concentrations and composition of proanthocyanidins. Proanthocyanidins are known to accumulate mainly before veraison, and their concentrations decreased during ripening (Downey et al. 2004; Koyama et al. 2008). The ABA treatment did not affect the decline of these compounds during ripening.

The in vitro berry culture was convenient for studying berry ripening, in particular, coloring in the berry skins (Kataoka et al. 1992; Hiratsuka et al. 2001). Unbiased experimental results without potential variables within the clusters are obtained. In addition, all detached berries were uniformly treated. The drawback of this system was that the assay could not be applied for long-term experiments because of the difficulty of completely sterilizing the surface of the berries without affecting their viabilities. The ABA concentrations in the berry skins on the ABA medium were remarkably higher than those in the control (0.61 $\mu g \cdot g^{-1}$ skin and 121.75 $\mu g \cdot g^{-1}$ skin in the control and ABA-treated berries, respectively, at Day 3; 0.43 $\mu g \cdot g^{-1}$ skin and 100.34 $\mu g \cdot g^{-1}$ skin in the control and ABA-treated berries, respectively, at Day 10). The soluble solid concentrations in the berries cultured in vitro increased from 3 to 10 days after placement on the plate, while the titratable acid concentrations decreased (Supplemental Table S2). These trends were close to those in the berries grown on the vine during ripening. When the ABA solution was added to the culture medium, pigment accumulation was accelerated, and, at Day 3, all berries on the ABA medium were colored, while 25% of the berries placed on the control medium were not colored at the time. The anthocyanin concentrations increased from Day 3 to Day 10 (Supplemental Table S3). When ABA was added, the anthocyanin concentrations were about 3.4-fold higher than those of the control at Day 10. These values were comparably high with those in the ABA treated berries grown on the vine at 14 DAV. Although there were differences between the anthocyanin composition in the control berries cultured in vitro and that of those grown on the vine, ABA addition to the culture medium had a similar effect on the anthocyanin composition to that was observed in the berries grown on the vine under ABA treatment (Table 1; Supplemental Table S3). The concentrations of flavonol and resveratrol were higher in the ABA-treated berries than in the control berries.

Reflecting the differences between anthocyanin concentrations in the ABA-treated berry skins and the control ones, the absorbance at 520 nm and color density in the wines that were vinificated from

the ABA-treated grapes were about 3-fold higher than those from the control berries (Supplemental Table S4). The total phenol concentrations measured by A_{280} were also higher in the wine made from grapes treated with ABA than that made from the control grapes.

Effects of ABA treatments on the transcriptome in the berry skins grown on the vine and cultured in vitro

The mRNA expression profiles in the ABA-treated and control berries grown on the vine as well as those cultured in vitro were compared using the Affymetrix *Vitis vinifera* Genechip. The average background was 48.0, and the average noise (RawQ) was 1.7. These values were comparable across arrays, as recommended by the Genechip Operating Software Users Guide. After pre-processing and normalization, perfect match intensities were consistent across all arrays with no apparent outlying arrays (Supplemental Fig. S1). Digestion curves describing trends in RNA degradation between the 5' end and the 3' end in each probe set were examined, and all 20 arrays proved comparable (Supplemental Fig. S2). Correlations among biological replicates were good: the Pearson correlation coefficients ranged from 0.978 to 0.995. The average present call rate across all 20 arrays was 72.7%.

In the ABA-treated berries grown on the vine at 14 and 28 DAV, 131 and 62 probe sets were significantly upregulated, respectively, with expression ratios more than twice that of the control, while 157 and 76 probe sets were significantly downregulated with expression ratios less than half that of the control. The number of up- or downregulated transcripts under ABA treatment decreased with time from 14 DAV to 28 DAV. The decrease in the number of the differentially expressed transcripts may reflect the lesser importance of the role played by ABA in the berry skins at later stages during ripening. Eighty-one probe sets overlapped between the differentially expressed transcripts on 14 DAV and those on 28 DAV. Thus, 345 probe sets showed significantly different abundance by more than twice or less than half relative to the control at least at one stage under ABA treatment (Supplemental Table S5). On the other hand, in the skins of ABA-treated berries cultured in vitro, 471 probe sets were significantly upregulated, with expression ratios more than twice that of

the control, and 555 probe sets were significantly downregulated, with expression ratios less than half that of the control at Day 3. At Day 10, 453 probe sets were upregulated, and 546 probe sets were downregulated. No dramatic changes in gene expression between Day 3 and Day 10 were observed. More than half of the up- or downregulated transcripts at days 3 and 10, 543 probe sets, overlapped. Thus, 1,482 probe sets showed significantly different transcript abundance by more than twice or less than half relative to the control at least at one stage under ABA treatments (Supplemental Table S6). A significant number of transcripts in the differentially expressed transcript sets in the berries grown on the vine at 14 DAV were included in those of the in vitro-cultured berries at Day 10 ($p = 5.70\times10^{-12}$, Fig. 2a). The difference of treatment intensity, as well as the reduction of the potential variables involved in the field experiment, was a probable reason for the much larger number of the differentially expressed transcripts in the berry skins cultured in vitro.

Within the functional categories from the Munich Information Center for Protein Sequences (MIPS, ver. 2.0) catalog, 'Metabolism','Cell rescue, defense, and virulence', and 'Interaction with environment' were significantly overrepresented in both up- and downregulated transcript sets in the berry skins grown on the vine at 14 DAV relative to the population of these categories in the entire Genechip sequences (Fig. 1). On the other hand, 'Protein fate' and 'Unclassified protein' were significantly underrepresented. 'Energy' was more populated in the downregulated transcripts than in the upregulated ones. When the differentially expressed transcripts in the berries grown on the vine were compared with those in the in vitro-cultured berries, the only differences in relative gene frequencies of the functional categories were the higher relative frequencies of 'Energy' and 'Cell rescue, defense, and virulence,' and the lower frequencies of 'Communication/signaling' in the berry culture at Day 10. The other categories had similar relative gene frequencies (Fig. 2b; Supplemental Fig. S3). Interestingly, although cultured berries were not supplied with nutrients except for sucrose, the differentially expressed transcripts between ABA treated- and untreated berries cultured in vitro and those on the vine showed a considerable degree of similarity regarding their proposed function.

Using K-means clustering, the temporal changes in the relative expression of the 345 differentially expressed transcript sets under ABA treatment in the berries grown on the vine were classified into six patterns, clusters I – VI (Fig. 3). Cluster III contains 32 transcripts whose relative expressions

continuously increased after ABA treatment until 28 DAV. Clusters I and II, which contained 72 and 57 transcripts, respectively, showed patterns of early induction under ABA treatment. While the relative expression levels of the transcripts in cluster I were kept relatively constant from 14 DAV to 28 DAV, those in cluster II decreased more remarkably. The transcripts related to anthocyanin biosynthesis were all included in cluster II. By contrast, clusters IV and V, which contained 90 and 68 transcripts, respectively, showed patterns of early reduction under ABA treatment. While the relative expression levels of the transcripts in cluster IV remained relatively constant from 14 DAV to 28 DAV, those in cluster II increased more remarkably. Most of the transcripts related with photosynthesis were included in cluster IV. The relative expressions of 26 transcripts (cluster VI) continuously decreased under ABA treatment. Several gene families exhibited multiple expression patterns under ABA treatment. For example, chitinase, the proline-rich cell wall protein, and the thaumatin-like proteins were present in clusters of both down- and upregulated transcripts.

The differentially expressed transcripts in the berries grown on the vine and those cultured in vitro included a significant number of transcripts in reactive oxygen species (ROS)-detoxification enzymes, the proline and sugar biosynthesis-related proteins, the pathogen- and disease-resistance related proteins, heat-shock proteins, protease inhibitors, and LEA proteins. These ABA-modulated transcripts were in common with those in other plant species (Seki et al. 2002; Rabbani et al. 2003; Buchanan et al. 2005; Matsui et al. 2008).

Deluc et al. (2009) recently reported water deficit increased ABA, proline, sugar, and anthocyanin concentrations in the berries of Cabernet Sauvignon. Consistently, the induction of the genes with function related to the biosynthesis of anthocyanin and these osmolytes was also observed in this study. High ABA concentrations particularly in the cultured berry skins may not necessarily reflect the active uptake and subsequent metabolism and signaling of ABA, as its transport and cellular and subcellular distribution within the berry tissues may be different from those of endogenous ABA. However, the overlapping with water stress inducible genes indicates that the transcripts up- and downregulated by exogenous ABA treatment are potentially regulated by endogenous ABA in vivo.

About half of the transcripts (155 transcripts) within modulated transcripts at least at one stage in the berries grown on the vine under ABA treatment were identical to the previously reported

ripening-specific gene set (Pilati et al. 2007) (Supplemental Table S5). Interestingly, most transcripts (54 of 55 transcripts) that showed the ripening-specific induction (profile 4 and 5) were upregulated under ABA treatment in this study, whereas most transcripts (75 of 76 transcripts) that showed ripening-specific reduction (profile 1 and 8) were downregulated under the treatment.

Some of the specific details of the major metabolic pathway influenced by ABA treatment are presented in the following section. A list of selected transcripts differentially expressed under ABA treatment and discussed in the text is shown in Table 2, while the complete lists of these transcripts are provided in Supplemental Tables S5 and S6.

Transcripts associated with metabolism of anthocyanin and other phenolics

The structural genes in the phenylpropanoid and flavonoid pathways: two phenylalanine ammonia-lyase (PAL) (1619642_at, 1613113_at), chalcone synthase (CHS) (1617019_at), flavanone-3-hydroxylase (F3H) (1606435_at), and the transcription factor regulating the anthocyanin biosynthetic pathway: *VvMYBA1&2* (1615798_at) (Kobayashi et al. 2004; Walker et al. 2007) were upregulated at 14 DAV (cluster II). The relative expression patterns of these genes were consistent with the rapid induction of anthocyanin concentration, indicating that anthocyanin biosynthesis is regulated predominantly at the level of transcription, consistent with the other reports (Davies and Schwinn 2003). In addition, other *V-myb myeloblastosis viral oncogene homolog (avian; MYB)* transcriptional regulators considered to be involved in the phenolic biosynthesis: a homolog of *GHMYB8* (1606690_at), *VvMYBPA1* (1616094_at), also showed increased transcript abundance (Elomaa et al. 2003; Bogs et al. 2007).

Serine carboxypeptidase-like proteins were considered to have function of flavonoid acylation (Tohge et al. 2005; Ikegami et al. 2007). The temporal changes in relative expressions of two serine carboxypeptidase-like protein genes (1616850_at, 1621067_at) under ABA treatment were related with the accumulation of anthocyanin (cluster II).

A homolog to the multidrug and toxic compound extrusion (MATE) family transporter *TRANSPARENT TESTA* 12 (*AtTT12*) (1607560_at) showed increased transcript abundance with a

similar expression pattern to that of several anthocyanin biosynthetic genes (cluster II). AtTT12 protein was reported to function on the transport of cyanidin 3-*O* glucoside and epicatechin 3'-*O*-glucoside from the cytosol into the vacuole in the seed epidermal cells (Marinova et al. 2007; Zhao and Dixon 2009). *VvGST1* (1609330_at), which was characterized as anthocyanin transporter from grape cell suspension cultures (Conn et al. 2008), was remarkably upregulated by more than 50-fold at 3 and 10 days after ABA treatment in the berries cultured in vitro, although this transcript did not show the modulated transcript abundance in the berries grown on the vine.

In the berries cultured in vitro, several stilbene synthases had remarkably increased transcript abundance, corresponding to the increase in the resveratrol concentrations in the ABA-treated berries (Supplemental Table S3).

Transcripts associated with the osmotic response

The biosynthesis of osmolytes, such as amino acids and sugars was considered to be one of the plant responses to adjust the osmotic pressure under drought conditions (Bray 1997). It was proved that part of these drought responses were induced through the production of ABA (Seki et al. 2002; Rabbani et al. 2003; Matsui et al. 2008). In this study, the exogenous ABA treatment upregulated several transcripts related to the biosynthesis of amino acids, sugars, and other osmolytes as well as their transport.

In the skins of berries grown on the vine and cultured in vitro, putative vacuolar glucose transporter (1613408_at) was induced under ABA treatment. This gene exhibited one of the greatest changes in transcript abundance (a higher than 5-fold increase at 14 DAV) in the berries grown on the vine. This vacuolar transporter was reported to function in the transport of glucose and fructose through tonoplast to adjust the osmotic pressure (Aluri and Büttner 2007). Furthermore, in the berries cultured in vitro, ABA treatment increased the abundance of the transcript encoding neutral invertase (1620628_at) and, particularly, sucrose synthase (1619223_s_at) by more than 14-fold, indicating that the sucrose was either cleaved into glucose and fructose by cell wall neutral invertase or degraded into UDP glucose and fructose by sucrose synthase, followed by hexose uptake and/or

metabolism. In consistent with our results, Kataoka et al. (1992) and Hiratsuka et al. (2001) reported the increased concentrations of hexoses, but not sucrose, by exogenous ABA application in the skins of grape berries. Sugars are reported to act as signaling molecule in the plant, and regulation of anthocyanin biosynthesis by sugars was observed in *Arabidopsis* (Teng et al. 2005; Sofanelli et al. 2005).

A gradual increase in the transcript abundance of galactinol synthase (1616448_at) in the ABA-treated berries over the control ones was observed (cluster III). This enzyme produces galactinol (disaccharide) from UDP-galactose. The importance of this gene for stress tolerance in *Arabidopsis* was reported (Taji et al. 2002).

ABA treatment coordinately reduced the transcript abundance of several classes of aquaporins in the berries cultured in vitro, indicating another osmotic response occurred through an ABA-dependent pathway.

Transcripts associated with photosynthesis and antioxidant metabolism

Most of the differentially expressed transcripts assigned to ‘photosynthesis’ and ‘accessory proteins of photosynthetic electron transport’ were downregulated under ABA treatment (cluster IV). Transcripts encoding specific photosystem I and II components (1607516_at, 1622534_at, 1621532_at) and several light-harvesting chlorophyll a/b-binding protein components (1619903_at, 1612273_at, 1618116_s_at, 1609044_at, 1608311_at, 1616560_at, 1615822_at, 1616940_s_at, 1611464_at) were included in cluster IV. In the berry culture, ABA treatment also downregulated the ribulose-1,5-bisphosphate carboxylate oxygenase activase (Rubisco activase; 1616918_s_at) concomitantly with the downregulation of ATP synthase component genes (1615602_at, 1614676_s_at). Furthermore, chlorophyll synthases (1620009_at, 1620634_at, 1621374_at) were reduced by the ABA treatment in the berry culture, consistently with a report that ABA regulated a decrease in chlorophyll content in the grape berries (Geny et al. 2004). The decreased abundance of many transcripts associated with photosynthesis in the berry skins with ripening was reported and considered to reflect the fact that the berries switch from a photosynthetically active status to a sink

status (Terrier et al. 2005). Thus, the results in this study show that the ABA treatment advances this change.

The reduction of the ability of the photosynthetic apparatus causes an imbalance between the generation and utilization of electrons that produce deleterious ROS within the cells (Reddy et al. 2004). In this study, the transcripts of several ROS-scavenging enzymes, such as peroxiredoxin, peroxidase, glutathione transferase, thioredoxin, glutaredoxin and metallothioneins, were differentially expressed under ABA treatment. The accumulation of anthocyanin was also considered to be a plant response to scavenge ROS in *Arabidopsis* (Nagata et al. 2003).

Transcripts associated with biotic and abiotic stress responses

The ABA treatment induced many stress-inducible genes in common with various plant species under ABA- and water-deficit treatment (Bray 1997; Seki et al. 2002; Rabbani et al. 2003; Buchanan et al. 2005; Umezawa et al. 2006; Cramer et al. 2007; Grimplet et al. 2007).

The main pathogen-related proteins in the grapes, such as chitinases (1620505_at, 1614551_at, 1611876_s_at, 1617430_s_at), thaumatin (-like) proteins (1622374_at, 1616695_s_at, 1606517_at, 1616413_at), and beta-1,3-glucanase (1620496_at, 1615595_at) showed remarkably increased transcript abundance under ABA treatment. Many of the pathogen-related proteins have been documented as prevalent proteins in ripe berries (Sarry et al. 2004). Interestingly, some of them are induced in a ripening-associated manner and even used as markers for the progress of ripening (Robinson et al. 1997; Tattersall et al. 1997; Davies and Robinson 2000; Pilati et al. 2007), suggesting that these proteins function to restrict pathogen invasion rather than repair damage, or they might have another unknown function. A beta-glucanase from the pulp tissue of banana that may be involved in ripening and tissue softening has been characterized (Peumans et al. 2000).

Dehydration was reported to induce proteins that probably function in stress tolerance, such as LEA proteins in other plants through an ABA-dependent pathway (Seki et al. 2002; Rabbani et al. 2003; Buchanan et al. 2005). Although the precise mechanism is still unknown, these proteins have been proposed to function as chaperone-like protective molecules and act against cellular damage

(Umezawa et al. 2006). In the berries grown on the vine, the gene expression of dehydrin 1a (1621592_s_at) encoding a group 2 LEA protein (Xiao and Nassuth 2006), as well as three LEA proteins (1617579_at, 1618211_at, 1620276_at), were induced by the ABA treatment. Dehydrin 1a showed one of the greatest changes in transcript abundance (6.2-fold induction at 14 DAV).

Furthermore, the induction of transcripts associated with protein recovery, such as proteases, protease inhibitors, and the heat-shock proteins was observed in the ABA treated berry skins, similar to the observation in the ABA-treated *Arabidopsis* plantlet (Seki et al. 2002; Matsui et al. 2008).

Transcripts associated with the lipid metabolism and cell wall modification

Not only the increased transcript abundance of acyl-CoA synthase (1610608_at, 1607174_at) as well as some lipases and esterases on the catabolic pathway of lipids (beta oxidation), but also the increased transcript abundance of ATP citrate lyase b-subunit (1609985_at, 1618362_s_at) and the fatty acid elongase-like protein (1609157_at, 1613811_a_at) on the biosynthetic pathway of fatty acids and lipids, suggest the activation of lipid metabolism in ABA-treated berries grown on the vine. These changes are possibly related to the modification of the cell membranes under the treatment.

In the ripening berry skins of Cabernet Sauvignon, changes in pectic polysaccharides of the cell walls and a decrease in neutral sugars, especially galactose, as well as an increase in the protein content at the end of the maturation period, were observed (Ortega-Regules et al. 2008). In this study, a pectin-modifying enzyme, polygalactosidase *VvPG1* (1620305_at), showed increased transcript abundance in ABA-treated berries grown on the vine, and another probeset (1614008_at) annotated as polygalactosidase *VvPG1* as well as pectin methyl esterase *VvPME1* (1619468_at) were upregulated in berries cultured in vitro, indicating the possible pectin modification (Barnavon et al. 2001; Nunan et al. 2001; Deytieux-Belleau et al. 2008). Furthermore, several proline-rich cell wall proteins were up- and downregulated under ABA treatment. One transcript upregulated (1616045_a_at) was reported to be a grape ripening-induced protein (GRIP13; Davies and Robinson 2000). These structural cell wall proteins was considered to be involved in providing additional support to the polysaccharide network in the cell walls by the formation of intermolecular cross-links.

Thus, the differential transcript abundances of cell wall proteins, as well as pectin modifying enzymes, may reflect the modification of cell walls occurring under ABA treatment.

Transcripts involved in regulatory processes

In this study, transcription factors up- or downregulated under ABA treatment belonged to the several classes; homeotic genes: homologs of ATHB-12 (1613946_s_at, 1609295_at) and YABBY-like transcription factor GRAMINIFOLIA (1612362_at); MADS-box protein family (1621420_at, 1621836_at); NAC (1606678_at, 1612448_at); MYB protein family (1611920_at, 1622064_at, 1619579_at) which include homologs to *VlMYBB1-2* and *VlMYBC*.

The transcripts associated with phytohormone metabolism and responses

Considerable cross-talks of ABA signaling with phytohormone signalings, in particular, auxin- and ethylene signaling, were suggested in this study.

The auxin content in deseeded berries was reported to be maximized during early berry development and then decline to very low levels in ripe berries, consistently with the role of this hormone in cell division and expansion during early berry development (Inaba et al. 1976). All the transcripts, except one, associated with auxin response were downregulated under ABA treatment. The transcription of the auxin response factor 5 (1612180_at) and Aux/IAA proteins (1621754_at, 1620512_at), which regulate the expression of auxin responsive genes, were already downregulated at 14 DAV (cluster IV or V). These results were in consistent with the report showing that the decrease in indole acetic acid levels in the ripening grape berry after ABA treatment (Geny et al. 2004).

Although only weak changes in endogenous ethylene levels around veraison have been described in grape berries, some studies showed that ethylene affected the physiological processes during ripening of berries (Tesniere et al. 2004; Chervin et al. 2004, 2008). Several transcripts associated with the ethylene metabolism and responses were affected in ABA-treated berries cultured in vitro.

The enzymes associated with methionine metabolism, such as homosysteine *S*-methyltransferase (1622196_at) and *S*-adenosylmethionine synthetase (1619909_at) as well as three ACC oxidases (1616698_at, 1622147_at, 1609995_s_at) showed increased transcript abundance, indicating the increased ethylene biosynthesis. Furthermore, the increased transcript abundance of the ER6 protein (1618213_at) and ethylene-responsive transcriptional coactivator-like protein (1621552_at), as well as the differential transcript abundance of 6 transcription factors belonging to ERF/AP2 families, suggested the activation of ethylene signaling.

In the berries cultured in vitro, the auto-induction of ABA biosynthesis in response to exogenous ABA application was suggested by the induction of nine-cis-epoxycarotenoid dioxygenase 4 (*NCED4*) (1607029_at) which encodes the rate limiting step in ABA biosynthesis. Similarly, the positive feedback control of ABA biosynthesis was reported in peanut (Wan and Li 2006).

Conclusions

ABA is a multi-functional phytohormone. In ripening grape berries, the concentration of ABA increases rapidly, and exogenous ABA induces anthocyanin and hexose accumulation in the skins. Though ABA has been considered to be a ripening-related hormone of grape, its function during ripening was still not clear. In this study, transcriptomic analysis showed that about half of ABA-induced transcripts in the berries grown on the vine were identical to the previously reported ripening-specific genes. The genes reported to show ripening-specific induction or reduction were up- or downregulated, respectively, which indicates that ABA advanced a considerable part of the ripening process in the berry skins. These ABA- and ripening-induced genes include genes associated with stress response, such as beta-1,3-glucanase, chitinases, thaumatins, and LEA proteins, as well as those associated with cell wall modification, such as polygalacturonase PG1, and proline-rich cell wall proteins, in addition to anthocyanin biosynthetic gene. On the other hand, the ABA- and ripening-reduced genes include those associated with photosynthesis, such as chlorophyll a/b binding protein and photosystem components, those associated with auxin response, such as auxin response factor 5, AUX/IAA proteins, as well as proline-rich cell wall protein genes.

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Table 1 Effect of ABA treatment on the phenolic concentration and composition in the skins of Cabernet Sauvignon grape berries grown on the vine

	0 DAV	14 DAV		28 DAV		37 DAV	
		control	ABA	control	ABA	control	ABA
Total anthocyanins (mg g-1 skin)	0.97±0.06	3.29±0.56	8.74±0.43*	3.07±0.48	8.13±1.87*	3.82±0.40	8.96±0.54*
%3-G	58.30±8.58	51.33±0.46	56.95±0.56*	50.87±1.62	58.12±1.11*	51.91±0.38	56.98±0.84*
%3Ac-3-G	33.53±8.56	35.04±0.47	32.67±0.05*	34.29±0.86	31.22±0.59*	33.52±0.23	31.12±0.21*
%pC-3-G	6.74±0.44	11.99±0.08	9.24±0.51*	13.09±0.74	9.57±0.50*	12.8±0.27	10.71±0.78*
%Dp-3-G	8.06±2.06	3.63±0.17	7.70±0.57*	3.07±0.29	6.94±0.86*	2.97±0.02	5.74±0.45*
%Cy-3-G	2.51±0.87	0.58±0.02	1.02±0.19*	0.55±0.09	0.94±0.22*	0.62±0.02	0.88±0.17
%Pt-3-G	5.88±1.1	3.44±0.05	5.47±0.24*	3.00±0.22	5.11±0.42*	3.00±0.07	4.58±0.17*
%Pn-3-G	7.98±1.92	4.33±0.22	4.52±0.37	3.96±0.07	4.47±0.37	4.39±0.41	4.56±0.31
%Mv-3-G	33.87±3.38	39.35±0.28	38.24±0.84	40.29±1.01	40.66±0.73	40.94±0.33	41.22±0.39
%Dp-Ac-3-G	1.40±1.23	0.77±0.06	2.00±0.20*	0.64±0.00	1.63±0.20*	0.60±0.01	1.35±0.14*
%Pt-Ac-3-G	9.55±12.72	1.08±0.05	2.08±0.13*	0.88±0.03	1.71±0.13*	0.82±0.02	1.44±0.09*
%Pn-Ac-3-G	3.25±0.59	2.43±0.07	2.68±0.13*	2.22±0.06	2.37±0.08	2.39±0.11	2.33±0.07
%Mv-Ac-3-G	19.32±2.59	30.76±0.41	25.91±0.47*	30.55±0.89	25.51±0.96*	29.72±0.29	26.00±0.19*
%Pn-pC-3-G	0.91±0.08	0.81±0.09	0.80±0.02	0.84±0.08	0.79±0.02	0.99±0.13	0.90±0.04
%Mv-pC-3-G	5.83±0.48	11.17±0.10	8.44±0.50*	12.25±0.68	8.78±0.48*	11.81±0.30	9.81±0.74*
Flavonols (mg g-1 skin)	0.07±0.02	0.20±0.07	0.38±0.04*	0.18±0.05	0.37±0.05*	0.26±0.02	0.43±0.06*
Cinnamic acids (mg g-1 skin)	0.39±0.02	0.35±0.02	0.33±0.04	0.28±0.02	0.25±0.06	0.26±0.02	0.26±0.01
Proanthocyanidins (mg g-1 skin)	28.07±1.22	27.68±1.74	25.05±2.39	19.85±1.96	17.4±1.06	22.28±1.00	21.52±1.01
%Catechin	3.58±0.58	3.95±0.25	3.50±0.60	3.56±0.34	3.63±0.11	3.67±0.20	3.47±0.23
%Epicatechin	53.69±1.57	55.87±3.20	54.31±2.18	56.86±2.27	57.13±1.35	56.48±2.00	55.37±0.50
%Epigallocatechin	40.81±1.91	37.96±3.58	40.22±1.69	37.50±2.51	37.12±1.25	37.70±2.34	39.02±0.32
%Epicatechin-gallate	1.92±0.11	2.22±0.35	1.97±0.19	2.08±0.21	2.12±0.13	2.15±0.25	2.14±0.30

* $p<0.05$; t-test, the values of the ABA-treated berries were significantly different from those of the control.

Table 2 Selection of genes modulated under ABA treatment in the berry skins of Cabernet Sauvignon grown on the vine and/or cultured in vitro

Probeset ID	Uniprot ID	Gene name description	Vine		Berry culture	
			14 DAV	28 DAV	Day 3	Day 10
Phenolic metabolism						
1613113_at	Q6UD65	Phenylalanine ammonia lyase (EC 4.3.1.5)	**1.21**	0.41	0.60	0.73
1619642_at	Q94C45	Phenylalanine ammonia-lyase 1 (EC 4.3.1.5)	**1.52**	0.46	0.93	0.96
1617019_at	O80407	Chalcone synthase (EC 2.3.1.74)	**1.22**	0.47	**1.08**	**1.03**
1607739_at	P41090	Naringenin,2-oxoglutarate 3-dioxygenase (EC 1.14.11.9) (Flavonone-3-hydroxylase)	**1.06**	0.38	0.48	0.56
1606435_at	Q6R3N2	Flavanone-3-hydroxylase	**1.16**	-0.32	1.68	0.71
1621067_at	Q8W3C8	Putative serine carboxypeptidase	**1.31**	0.43	0.93	**1.37**
1616850_at	Q9LKY6	Glucose acyltransferase	**2.65**	0.61	**1.84**	**2.05**
1609330_at	Q6YEY5	Glutathione S-transferase	0.01	-0.25	**8.19**	**5.84**
1615798_at	Q8L5P1	Myb-related transcription factor VvMYBA1&2	**1.17**	0.29	0.82	-0.06
1616094_at		VvMYBPA1	0.69	0.46	**1.44**	**1.39**
1606690_at	Q70RD2	GHMYB8	1.54	**1.67**	0.30	**-4.53**
Osmotic response						
1613408_at		Vacuolar glucose transporter	**2.47**	1.20	**2.12**	**1.98**
1620628_at	Q9ZR47	Neutral invertase (EC 3.2.1.26)	0.09	0.17	0.87	**1.52**
1619223_s_at	Q9SLS2	Sucrose synthase	0.54	0.83	**3.88**	**3.39**
1614939_at	Q9MBD7	NAD-dependent sorbitol dehydrogenase	**1.03**	-0.64	**1.40**	0.13
1616448_at	Q9SPE1	Galactinol synthase (Fragment)	0.85	**1.77**	-2.29	-1.15
1617035_s_at	Q9XGN4	Galactinol synthase, isoform GolS-1 (EC 2.4.1.123)	0.30	-0.14	**2.56**	1.74
Photosynthesis						
1621532_at	Q84QE6	Photosystem I reaction center subunit X psaK	**-1.18**	**-1.20**	0.18	-0.36
1622534_at	Q84U30	Photosystem I-N subunit	**-1.07**	-0.87	**-1.50**	-1.14
1607516_at	Q9LRC4	Oxygen evolving enhancer protein 1 precursor	**-1.01**	**-1.03**	-0.62	-1.49
1619903_at	Q40512	Photosystem I light-harvesting chlorophyll a/b-binding protein	**-1.07**	**-1.02**	0.81	-0.74
1612273_at	P08221	Chlorophyll a-b binding protein of LHCII type I, chloroplast precursor (CAB) (LHCP) (Fragment)	-0.87	**-1.12**	0.45	-0.21
1611464_at	Q9XF85	Lhca5 protein	**-1.97**	-2.12	**-2.06**	-1.23
1616940_s_at	Q7M1K9	Chlorophyll a/b-binding protein (cab-11)	**-1.63**	**-1.06**	0.74	-0.51
1615822_at	Q9XQB1	LHCII type III chlorophyll a/b binding protein	**-1.55**	**-1.30**	-0.35	-0.58
1616560_at	Q84WT1	Putative light-harvesting chlorophyll a/b binding protein	**-1.48**	**-1.87**	**-2.35**	-1.31
1618116_s_at	Q32291	Chlorophyll A/B binding protein precursor	**-1.13**	**-1.01**	**1.45**	-0.56
1620009_at	Q38833	G4 protein (Chlorophyll synthetase)	-0.17	-0.06	-0.98	**-1.35**
1620634_at	Q7XC03	Putative chlorophyll synthase	-0.24	-0.26	**-1.05**	-0.02
1621374_at	Q5W6H5	Putative chlorophyll synthase	-0.18	0.02	**-1.11**	**-1.06**
1616918_s_at	Q40281	Ribulose bisphosphate carboxylase/oxygenase activase, chloroplast precursor (RuBisCO activase)	-0.65	-0.49	**-1.11**	-0.53
1614676_s_at	Q68S21	ATP synthase alpha chain	-0.26	-0.41	-0.63	**-1.08**

1615602_at	P29790	ATP synthase gamma chain, chloroplast precursor (EC 3.6.3.14)	-0.44	-0.57	**-1.29**	-0.84
Biotic and abiotic stress response						
Pathogen and disease resistance related proteins						
1611552_at	P29060	Acidic endochitinase precursor (EC 3.2.1.14)	**3.94**	**2.78**	**3.07**	**1.23**
1617430_s_at	P51613	Basic endochitinase precursor (EC 3.2.1.14)	**1.42**	**1.00**	**2.10**	1.50
1611876_s_at	Q945U2	Chitinase 3-like protein	**1.61**	**1.05**	**3.61**	**1.19**
1614551_at	Q6JX04	Chitinase-like protein (EC 3.2.1.14)	**1.69**	0.78	0.39	0.58
1622374_at	Q41350	Osmotin-like protein precursor	1.54	**1.67**	-0.02	0.38
1616695_s_at	Q9SPE0	Thaumatin	0.81	**1.17**	**3.03**	**2.48**
1606517_at	Q8LBL4	Putative thaumatin-like protein	**1.51**	1.18	**-1.16**	**-3.16**
1616413_at	O04708	VVTL1	**1.00**	**1.06**	**1.17**	**1.39**
1620496_at	Q8VY12	Putative beta-1,3-glucanase	**1.25**	0.20	-0.28	-1.35
1615595_at	Q9M563	Beta-1,3-glucanase	**2.05**	**1.81**	**5.77**	**6.04**
LEA protein						
1621592_s_at	Q3ZNL4	Dehydrin 1a	**2.63**	**2.11**	**1.29**	**2.17**
1617579_at	P13940	Late embryogenesis abundant protein D-29 (LEA D-29)	**1.75**	**1.08**	0.27	-0.69
1618211_at	Q39872	PGmPM3	**1.84**	**1.83**	**3.30**	**3.58**
1620276_at	Q9ZVC3	Putative embryo-abundant protein (At2g41380)	**1.25**	**1.40**	**3.47**	**4.50**
Lipid metabolism						
1610608_at	O48780	Putative beta-ketoacyl-CoA synthase	**1.41**	**1.33**	1.89	0.75
1609985_at	Q93YH3	ATP citrate lyase b-subunit (EC 4.1.3.8)	**1.25**	0.67	**1.48**	**1.78**
1609157_at	Q8LAF8	Fatty acid elongase-like protein (Cer2-like)	**1.58**	-0.11	**-2.72**	**-4.70**
1613811_a_at	Q9LZ72	Putative fatty acid elongase (At5g04530)	0.84	**1.08**	**1.13**	**1.33**
Cell wall metabolism						
1620305_at	Q94B15	Polygalacturonase PG1	**1.85**	2.35	1.97	1.49
1614008_at	Q94B15	Polygalacturonase PG1	1.64	0.80	**4.86**	2.98
1619468_at	Q94B16	Pectin methylesterase PME1	-0.29	-0.15	**2.03**	**2.56**
1607449_s_at	Q8LGR6	Proline-rich protein 1	**-1.61**	-0.18	-2.09	-2.41
1615201_at	Q5DNZ7	Putative proline-rich protein (Fragment)	**-1.51**	-1.11	**-1.37**	**-1.30**
1607162_s_at	Q96232	Proline-rich-like protein	**-1.46**	-1.40	-3.69	**-4.55**
1616045_a_at	Q9M4I0	Putative proline-rich cell wall protein (Fragment)	**1.23**	0.39	**-1.23**	-1.13
1607341_at	Q9FT04	Putative proline-rich protein APG isolog (Fragment)	**1.45**	0.08	**6.03**	**3.28**
1622481_x_at	Q39763	Proline-rich cell wall protein	**1.48**	0.74	-1.25	-1.90
Transcription factor						
1613946_s_at	Q9M276	Homeobox-leucine zipper protein ATHB-12	**1.49**	1.10	0.43	0.95
1612362_at	Q6SS00	YABBY-like transcription factor GRAMINIFOLIA	**-1.20**	**-1.01**	**-2.20**	**-2.25**
1621420_at	Q84LP9	MADS-box protein 6	**-1.15**	-0.69	-0.77	**-0.98**
1621836_at	Q8LLQ9	MADS-box protein 5	**-1.28**	-0.77	0.13	-1.77
1606678_at	Q8LF13	NAM, no apical meristem,-like protein	0.73	**1.30**	0.97	**2.09**
1612448_at	Q52QR0	NAC domain protein NAC6	**-1.67**	-0.01	0.71	0.15
1611920_at	Q9M9A3	F27J15.20 (Hypothetical protein) (MYB transcription factor)	-1.13	**-1.05**	**-1.07**	-1.52
1622064_at	Q8L5N7	Myb-related transcription factor VlMYBB1-2	0.24	0.33	1.68	**3.35**

1619579_at	Q8L5P0	Myb-related transcription factor VlMYBC (Fragment)	0.09	0.37	**1.29**	0.84
Hormone metabolism and response						
Auxin						
1612180_at	Q6L8T9	Auxin response factor 5	**-2.46**	**-1.44**	-0.62	-0.20
1620512_at	Q8RVH8	Aux/IAA protein	**-1.93**	-1.08	**-1.95**	**-4.03**
1612090_s_at	Q9ZRA4	Auxin-binding protein ABP19a precursor	**-1.09**	**-1.11**	-0.70	0.34
1620353_at	Q93WC4	Auxin-responsive protein IAA29	-0.33	**-1.25**	-1.19	-1.60
Ethylene						
1622308_at	Q9ZRC8	Similar to ACC synthase	-0.37	-0.80	**3.74**	**5.60**
1609995_s_at	Q8S932	1-aminocyclopropane-1-carboxylate oxidase (EC 1.14.17.4) (ACC oxidase)	-0.03	0.08	**1.81**	1.03
1622147_at	Q08507	1-aminocyclopropane-1-carboxylate oxidase 3 (EC 1.14.17.4) (ACC oxidase 3)	-0.04	-0.03	**1.76**	**1.19**
1618213_at	Q9SWV2	ER6 protein (Fragment)	-0.49	-0.21	1.05	**1.46**
1619585_at	Q75UJ4	ERF-like protein	0.07	0.29	**1.60**	**2.23**
1613698_at	Q6RZW8	Putative ethylene response factor 4	-1.13	-1.66	**-2.60**	**-2.16**
1611732_at	Q84Z02	Putative AP2/EREBP transcription factor	0.46	0.29	-2.20	**-1.98**
1621552_at	Q9LV58	Ethylene-responsive transcriptional coactivator-like protein (At3g24500)	0.44	0.13	**2.60**	**1.83**
ABA						
1619371_at	Q8LKV3	Beta-carotene hydroxylase	2.79	1.43	1.36	**2.39**
1607029_at	Q8LP14	Nine-cis-epoxycarotenoid dioxygenase4	0.76	0.68	**1.28**	1.33
1611998_at	Q8S4C2	Violaxanthin de-epoxidase	-0.26	-0.65	-0.33	**-1.52**
1610455_at	Q2PHF8	Carotenoid cleavage dioxygenase 1	-0.96	-0.30	**-1.71**	-1.81

The values represent $\log_2$-ratio of the averaged expression of ABA treatment versus that of control. Significant modulations of the gene expression are indicated by bold emphasis.

Figure legends

Fig. 1 Distribution of ABA-responsive transcripts according to their MIPS functional categories (MIPS 2.0) expressed in the berry skins of Cabernet Sauvignon at 14 DAV. One-hundred and thirty-one upregulated and 157 downregulated ABA-responsive transcripts are represented in a pie chart. The categories marked by a *black and a white diamond* were respectively over- and underrepresented in the gene set compared to the entire chip after statistical analysis (chi-square test, $p<0.05$). Significant differences between the distribution of the upregulated transcripts and that of the downregulated transcripts are indicated with a *star-shaped pentagon* (chi-square test, $p<0.05$). The unclassified category includes categories; classification not yet clear-cut, unclassified proteins, and other categories in which a few transcripts were assigned

Fig. 2 Comparison of the differentially expressed transcripts in the berries treated by ABA on the vine with those in the in vitro-cultured berries treated by ABA. **a** Venn diagram of the result. The differentially expressed transcripts in the berries grown on the vine at 14 days after ABA treatment and that in the in vitro-cultured berries 10 days after ABA treatment were compared. The significance of the overlaps (p values) between the two transcript groups were calculated by the Pearson's chi-square test. **b** Functional classification of the transcripts differentially expressed by ABA treatment under the above two conditions. Relative gene frequencies are calculated as the percentage of the number of the transcripts in a category against all differentially expressed transcripts in each treatment. Significant differences of the distribution of the transcripts between two treatments are indicated with a *star-shaped pentagon* (chi-square test, $p<0.05$).

Fig. 3 Classification of 345 ABA-responsive transcripts in the berry skins based on relative expression during ripening. Six temporal patterns of relative expression were visualized using k-means clustering with Pearson's correlation distance. The x-axis represents the time as days after veraison (treatment). The y-axis represents microarray $\log_2$-relative expression ratios in the ABA-treated berries against the control.

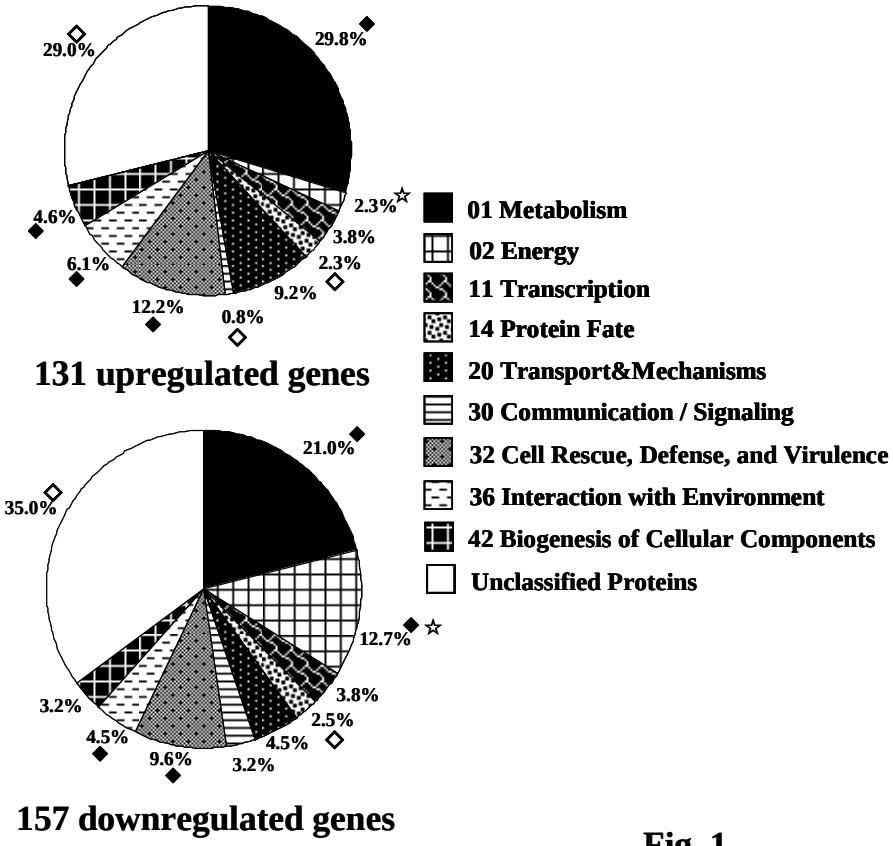

Fig. 1

Kazuya Koyama

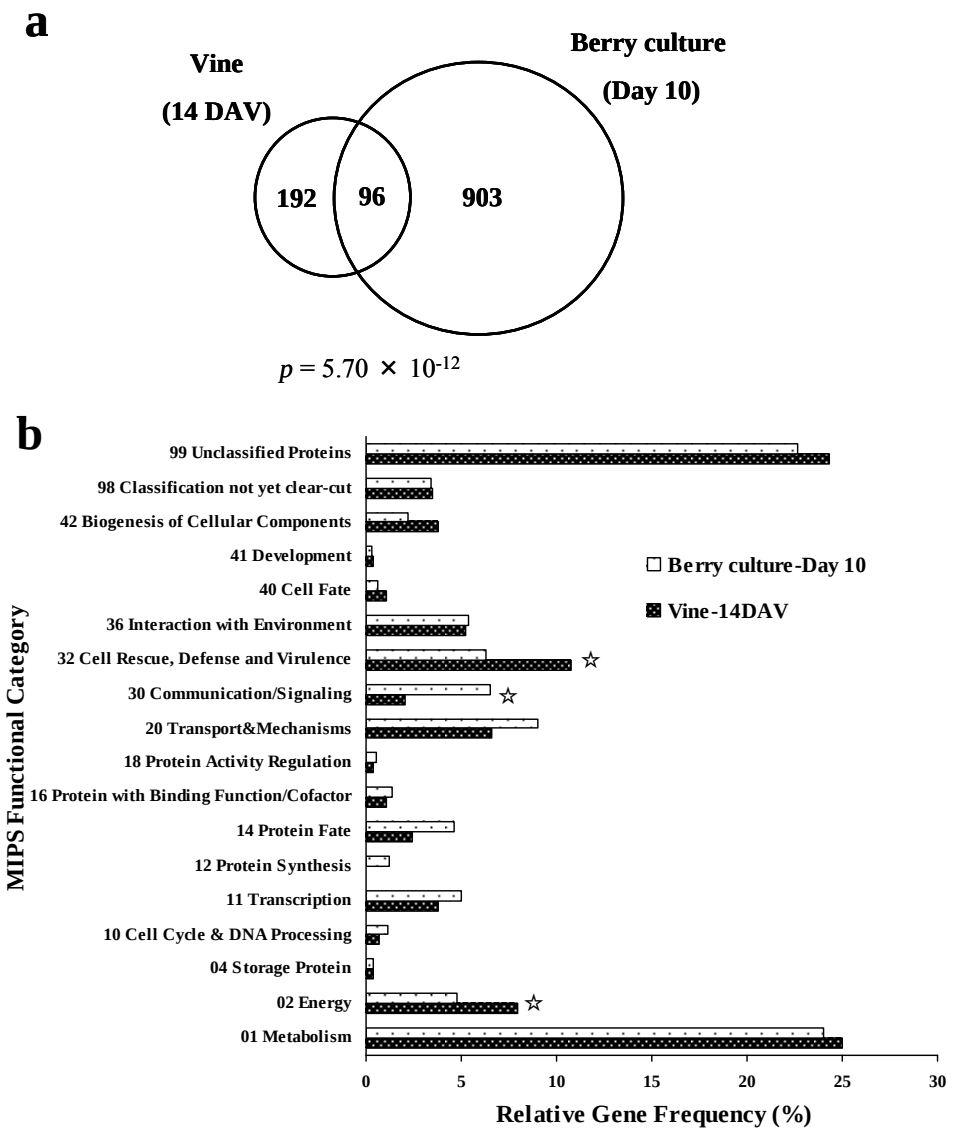

Fig. 2

Kazuya Koyama

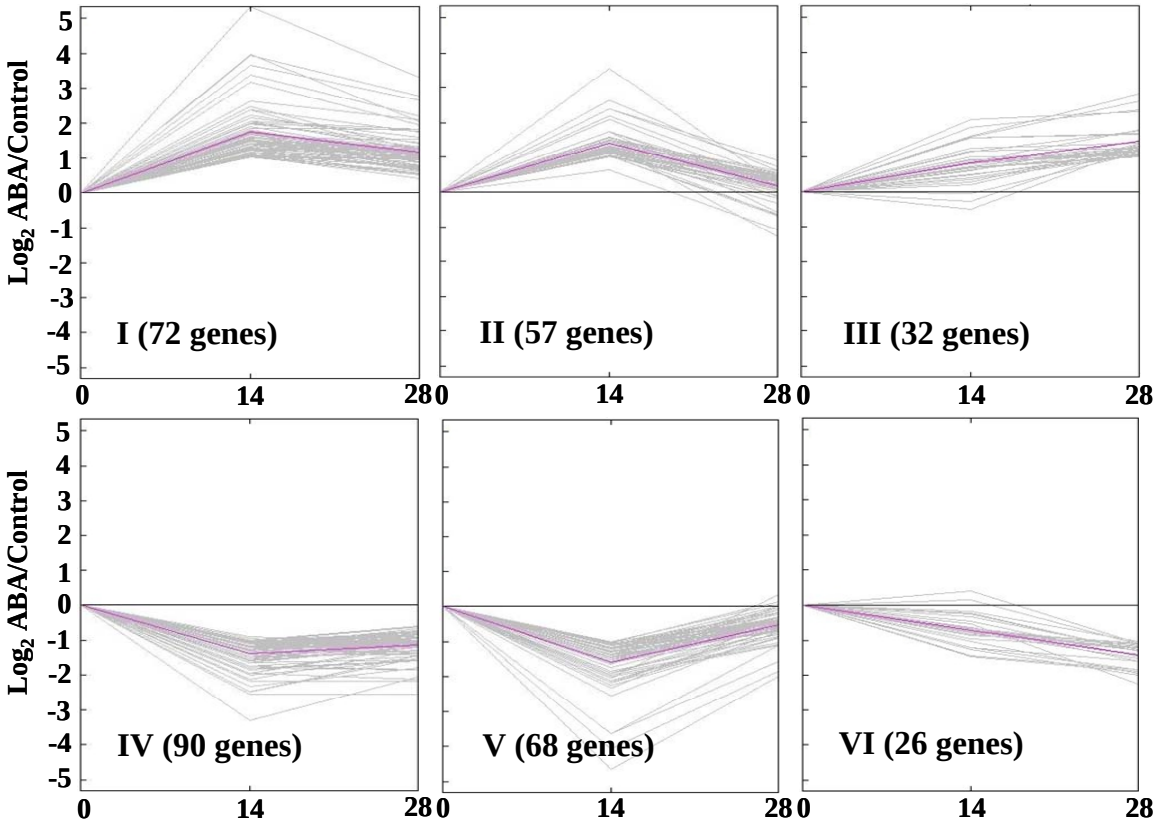

Fig. 3

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