Plant Physiology and Biochemistry 115 (2017) 286–297

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Relationship among color development, anthocyanin and pigment-related gene expression in 'Crimson Seedless' grapes treated with abscisic acid and sucrose

Daniela Olivares [a], Carolina Contreras [a], Victoria Muñoz [b], Sebastián Rivera [a], Mauricio González-Agüero [a], Julio Retamales [c], Bruno G. Defilippi [a, *]

[a] Instituto de Investigaciones Agropecuarias, INIA-La Platina, Santa Rosa 11610, Santiago, Chile
[b] Universidad Santo Tomás, Facultad de Ciencias, Ejército 217, Santiago, Chile
[c] INIA-La Platina, Universidad de Chile (Retiree), Chile

ARTICLE INFO

Article history:
Received 2 February 2017
Received in revised form
17 March 2017
Accepted 5 April 2017
Available online 6 April 2017

Keywords:
ABA
Anthocyanin
Chlorophyll
Color
Sucrose
Vitis vinifera

ABSTRACT

'Crimson Seedless' is one of the most important table grape varieties in Chile, but under certain environmental conditions, the fruit exhibits inadequate red color development, causing economic losses due to lower product quality. The use of plant growth regulators, such as abscisic acid (ABA) and ethylene, during development increases the anthocyanin content of the skin, improving the color of the berry. Recently, sucrose has been identified as a signaling molecule capable of regulating the expression of genes of the anthocyanin biosynthesis pathway. The aim of this study was to analyze the effect of application of ABA and/or sucrose on color development and their relationship with anthocyanin metabolism. Applications of ABA (400 ppm or 200 ppm) and/or sucrose (90 mM) were performed close to the *véraison* stage. During development and at harvest, quality attributes such as berry firmness, total soluble solids and titratable acidity were not affected by these treatments. Increased red color development was observed in fruits treated with ABA and/or sucrose, due to accumulation of anthocyanins. Fruits subjected to sucrose treatment showed higher levels of anthocyanins than untreated fruits but lower levels than fruits treated with ABA. Increased expression of genes involved in anthocyanin biosynthesis was observed in ABA- and sucrose-treated fruits compared to untreated fruits. Based on these findings, we demonstrated that sucrose improved fruit color development by increasing synthesis and accumulation of anthocyanins, thus allowing earlier harvests and improving table grape quality.

1. Introduction

Chile is the major worldwide exporter of table grapes, with approximately 860 thousand tons (equivalent to 1.306 million dollars) destined for markets in North America (mainly the USA), Europe and Asia (Bravo, 2014). The main varieties produced in Chile are 'Red Globe' (RG), 'Thompson Seedless' (TS) and 'Crimson Seedless' (CS), the latter one being the third most important table grape cultivar, with 21% of total production (Bravo, 2014). 'Crimson Seedless' is a late-season, bright red and seedless table grape cultivar that was initially bred in California (Mohsen, 2011; Ramming et al., 1995). Its berries are firm, crisp and have good flavor; skin color can vary from cherry red to black (Cameron, 2001; Dokoozlian et al., 1995). In Chile, as well as in other countries such as Australia, the USA and Italy, obtaining an adequate red color for CS fruit at harvest is a major problem (Singh Brar et al., 2008; Cameron, 2001; Ferrara et al., 2013). At least 30% of the fruit produced by this variety may remain on the vine unharvested due to inadequate red color development, with negative consequences for the grower (Dokoozlian et al., 1995). Skin color is a key quality attribute required by the consumer. Fruit coloration is due to the

Abbreviations: ABA, abscisic acid; RG, Red Globe; TS, Thompson Seedless; CS, Crimson Seedless; PAL, phenylalanine ammonia-lyase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavonoid 3-hydroxylase; DFR, dihydroflavonol 4-reductase; LDOX, leucoanthocyanidin dioxygenase; UFGT, UDP glucose:flavonoid-3-O-glucosyltransferase; DAV, days after *véraison*; TSS, total soluble solids; TA, titratable acidity; CIRG, color index of red grapes; FW, fresh weight; HPLC, high performance liquid chromatography; qRT-PCR, Quantitative real-time PCR; PCA, principal component analyses; Pn, peonidin; Mv, malvidin; Cy, cyanidin; Dp, delphinidin; Pt, petunidin; CC, color coverage of cluster.

* Corresponding author.
E-mail address: bdefilip@inia.cl (B.G. Defilippi).

http://dx.doi.org/10.1016/j.plaphy.2017.04.007
0981-9428/

presence of various pigments such as chlorophylls, carotenoids and anthocyanins (Singh Brar et al., 2008; Wei et al., 2011). The red color of the berry skin is a consequence of anthocyanin biosynthesis and accumulation in the cells (Yamane et al., 2006; Boss et al., 1996). The amounts of anthocyanins present in grape berry skins depend on the cultivar, seasonal conditions, phenological stage, and cultural practices (Fernández-López et al., 1998; Singh Brar et al., 2008; Liang et al., 2008). Poor color development in CS berry is likely a consequence of both summertime high temperatures, which inhibit the accumulation of anthocyanins, and a narrow day/night temperature range (Ferrara et al., 2013; Yamane et al., 2006; Dokoozlian et al., 1994; Spayd et al., 2002; Peppi et al., 2006). In red grapes, anthocyanin accumulation begins at the phenological stage of *véraison* and is regulated by a complex mechanism influenced by the plant hormone abscisic acid (Ferrara et al., 2015; Cantín et al., 2007).

Anthocyanins are synthesized by the phenylpropanoid pathway; several structural genes and encoding enzymes of this pathway have been well described (Villegas et al., 2016; Boss et al., 1996; Xie et al., 2011). The synthesis of anthocyanins begins with the conversion of phenylalanine to *4*-coumaroyl-CoA by the action of phenylalanine ammonia-lyase (PAL) (Xie et al., 2011). Chalcone synthase (CHS) forms naringenin-chalcone from 4-coumaroyl-CoA and malonyl-CoA (Xie et al., 2011). The naringenin chalcones formed are rapidly and stereospecifically isomerized to naringenin by chalcone isomerase (CHI), which is hydroxylated by flavonoid 3′-hydroxylase (F3H), transforming it into dihydrokaempferol (Xie et al., 2011; Del Valle et al., 2005; Boss et al., 1996). From this point, the synthesis of anthocyanins depends on oxidation and dehydration reactions catalyzed by dihydroflavonol 4-reductase (DFR) and leucoanthocyanidin dioxygenase (LDOX). Finally, anthocyanidins are glycosylated by glycosyl moieties from UDP-activated sugar donor molecules by the action of UDP glucose:-flavonoid-3-O-glucosyltransferase (UFGT); this gene is regulated by the MYBA1 transcription factor (Del Valle et al., 2005; Xie et al., 2011).

Gene expression and activation of enzymes for anthocyanin biosynthesis are influenced by three main factors: (i) climatic conditions, (ii) cultural practices and (iii) phenological stages. i) Anthocyanin accumulation is suppressed by high temperature and low light intensity (Spayd et al., 2002). For instance, temperatures of 30 °C inhibit anthocyanin accumulation in berries (Spayd et al., 2002; Yamane et al., 2006; Cantín et al., 2007). Grapes grown in warm regions develop less red color than those from cooler regions (Yamane et al., 2006; Peppi et al., 2006). ii) Careful canopy and crop management, as well as application of ethephon, optimize the color of CS grapes (Dokoozlian et al., 1994). To obtain homogeneous color throughout clusters, plant growth regulators (PGR), such as abscisic acid (ABA) and 2-chloroethylphosphonic acid (ethephon), are permitted under national regulations in some countries, such as the USA, Australia, Chile and Italy (Ferrara et al., 2015). Ethephon is usually applied to red table grapes to improve berry color, but its effects on color are inconsistent and can cause berry softening (Jensen et al., 1975, 1982; Szyjewicz et al., 1984; Peppi et al., 2006, 2007). Other studies showed that exogenous ABA treatment could increase skin anthocyanin concentrations in grapes, but the high cost of ABA has precluded the development of practical applications for viticulture (Peppi et al., 2006). iii) In regards to phenological stages, sugar is one of the main factors that contributes to color change. Sucrose is known to be an activator of gene expression for enzymes involved in anthocyanin biosynthesis, and sugar deficiency can delay pigmentation of fruits (Dai et al., 2014; Ferrara et al., 2015). Sugar begins to accumulate at a higher rate at the beginning of *véraison*, and some studies have reported that increases in the concentrations of both sugar and ABA were correlated with grape ripening (Zhang et al., 2009a). Sugars have been studied not only as an energy source but also as signaling molecules able to control gene expression (Lecourieux et al., 2013). The accumulation of anthocyanins in grapes has been shown to be stimulated by exogenous application of sugars to grape cell suspensions and tissue cultures, probably because of osmotic stress (Gambetta et al., 2010; Ferri et al., 2011).

The objective of this study was to analyze the effect of the application of abscisic acid and/or sucrose on color development and their relationship with anthocyanin and chlorophyll metabolism.

2. Material and methods

2.1. Plant material

Ten-year-old plants of table grapes cv. 'Crimson Seedless' were obtained from a commercial vineyard located in Los Andes (Aconcagua Valley, 32°52′27″ S and 70°38′26″ W), Chile. Plants were spaced 4 × 3 m grown on their own roots using an overhead trellis system and watered with drip irrigation. Vineyard operations such as plant fertilization, pest control, and other crop management were carried out according to local practices, without nitrogen additions during the season. A randomized block design with four blocks and six treatments was used. The treatments were Control; ABA (ProTone®, Valent BioSciences) at a concentration of 200 ppm (ABA200); ABA at a concentration of 400 ppm (ABA400); sucrose (Merck, Germany) 90 mM; ABA 200 ppm + sucrose 90 mM (ABA200 + sucrose); and ABA 400 ppm + sucrose 90 mM (ABA400 + sucrose). Four replicates per treatment were used, one replicate corresponded to one vine with 60 bunches. All treatments were applied directly to the 60 bunches with a hand-held sprayer at 13 days after *véraison* (DAV). *Véraison* was established when 50% of the berries in the cluster were soft, which actually corresponded to 51.11 ± 0.77% of berry softening. Grapes were sampled weekly, from the start of *véraison* (January 15, 2014, 0 DAV) until the time of harvest (March 20, 2014, 63 DAV). All grapes were harvested when 15% of the bunches from the control treatment had reached class 5 according to the visual color scale (Fig. 1). At each sampling time, 50 berries were obtained from 16 homogeneous clusters within each treatment. Immediately after sampling, grape berries were transported under refrigerated conditions to the Postharvest Laboratory at INIA-La Platina, Santiago (Chile) for evaluation of maturity parameters and metabolites and for molecular assays. For the molecular assays, twenty whole berries were frozen in liquid nitrogen and stored at −80 °C until further use.

2.2. Maturity parameters

Total soluble solids content (TSS) was measured with a manual temperature-compensated refractometer (ATC-1E, Atago, Tokyo, Japan) and the results were expressed as percentage (%). Titratable acidity (TA) was obtained by titrating 10 mL of juice from a representative sample of fruit with 0.1 N NaOH until neutralization of organic acids at a pH of 8.2. In this case, the results were expressed as a percentage of tartaric acid equivalents. Additionally, berry firmness was assessed by a Firmtech 2 texture analyzer (Bioworks, KS, USA), and the results were expressed in g mm^{-1}. For TSS and berry firmness, a total of 20 berries per replicate were considered, and for TA a composite sample was used for each replicate.

2.3. Color development and color quality assessments

Color development of sixteen clusters was assessed as percentage of color coverage by using a visual color scale with five

classes: class 1 = 100% green color, class 2 = <25% red color, class 3 = 25–50% red color, class 4 = 50–75% red color, and class 5 = 75–100% red color (Fig. 1). The color development of berries was also measured, using fifteen berries and a visual scale with 4 categories: class 1 = <25% red color, class 2 = 25–50% red color, class 3 = 50–75% red color, and class 4 = 75–100% red color (Fig. S1). The skin color quality was evaluated in fifteen berries with a reflectance colorimeter (CR-300, Minolta, Japan) using the CIELAB color system. Two equidistant color measurements were made around the equator of each berry according to Ferrara et al. (2013). L^*, a^*, b^*, C^* and h° units of color space were used (McGuire, 1992), where L^* is the lightness (0, black; 100, white); C^* is the Chroma, a measure of the intensity/purity of color (0 = achromatic); a* and b* are chromatic coordinates representing red-green and yellow-blue axes, respectively; and h° is the hue angle on the color wheel (0° = red; 90° = yellow; 180° = green; 270° = blue). From these data, the Color Index of Red Grapes (CIRG) (Carreño et al., 1995) was calculated as: CIRG = (180-h°)/(C*+L*). CIRG allows an objective definition of the external color in different red grape cultivars. Based on this index, the berries were classified into five categories: green-yellow (CIRG <2); pink (2 < CIRG<4); red (4 < CIRG<5); dark red (5 < CIRG<6); and blue-black (CIRG >6) (Carreño et al., 1996).

2.4. Anthocyanin analysis

Anthocyanins were extracted from 0.5 g of berry skin. The skin was ground with a mortar and pestle in liquid nitrogen and soaked in 5 mL of 75% (v/v) methanol for 20 min. The extract was then centrifuged at 10,000 rpm for 10 min at 10 °C. The supernatant was mixed with 20 mL of water and passed through a C18 solid-phase extraction cartridge (Bond elut C18-500 mg, Agilent, USA) preconditioned with pure methanol. After washing the cartridge with water, the anthocyanins were eluted with 2 mL of n-propanol and dried under nitrogen gas. Anthocyanins were suspended in 0.5 mL methanol/water (1/1, v/v) and analyzed by HPLC-DAD (Waters, Milford, Mass., USA) according to Peña-Neira et al. (2007). The calibration curves at 520 nm were obtained by injection of different volumes of standard solutions under the same conditions as the samples analyzed. The identification of anthocyanins was carried out by comparison of their spectra and retention times with those obtained by others authors (Fernández-López et al., 1998; Cantos et al., 2002; Peña-Neira et al., 2007; Singh Brar et al., 2008). Anthocyanins were quantified and expressed as mg/L of malvidin-3-glucoside (Sigma, Germany).

2.5. Chlorophyll analysis

Chlorophylls were extracted from 0.5 g of berry skin. The skin was homogenized with 80% (v/v) of cold acetone at −20 °C, placed on ice and left in the dark for 15 min before it was centrifuged at 10,000×*g* for 10 min at 4 °C according to standard procedures (Lichtenthaler and Wellburn, 1983). Absorbance was read at 645 and 663 nm on a Jenway 6715 UV/visible spectrophotometer (Bibby Scientific Limited, Staffordshire, UK). Chlorophyll *a* (Equation (1)), chlorophyll *b* (Equation (2)) and total chlorophyll (chlorophyll *a* + chlorophyll *b*), (Equation (3)) were calculated according to Arnon (1949). The results were expressed as μg chlorophyll per g of tissue fresh weight.

$$\text{Chlorophyll } a \text{ content (mg/gFW)} = ((12.7{*}A_{663}) - (2.6{*}A_{645}))/\text{FW}. \quad (1)$$

$$\text{Chlorophyll } b \text{ content (mg/gFW)} = ((22.9{*}A_{653}) - (4.8{*}A_{663}))/\text{FW}. \quad (2)$$

$$\text{Total Chlorophyll content (mg/gFW)} = \text{Chlorophyll } a + \text{Chlorophyll } b. \quad (3)$$

2.6. RNA isolation and cDNA synthesis

Total RNA was isolated from 1 g of frozen skin using a modified hot borate method (Gudenschwager et al., 2012). The quantity and quality of the RNA were assessed with a Qubit® 2.0 fluorometer (InvitrogenTM by Life Technologies, Singapore) by measuring the A260/280 ratio and by electrophoresis on a 1.2% formaldehyde-agarose gel. First-strand cDNA was obtained by reverse transcription with 2 μg of total RNA as the template, using M-MLV reverse transcriptase (Promega, Madison, WI) and oligodT primers according to standard procedures.

2.7. Quantitative real-time PCR assays

Five genes of the phenylpropanoid pathway were studied: *VvPAL* (phenylalanine ammonia-lyase, GenBank DQ887093.1); *VvCHS* (chalcone synthase, Gene ID 100263443); *VvCHI* (chalcone isomerase, Gene ID 100233078); *VvMYBA1* (transcription factor, GenBank AB097923.1); and *VvUFGT* (UDP glucose:flavonoid 3-o-glucosyltransferase, GenBank AF000371.1). Three genes of the chlorophyll degradation pathway were also studied: *VvCFL2* (chlorophyllase-2, NCBI Reference Sequence XM_002279249.3); *VvRCCR* (red chlorophyll catabolite reductase, NCBI Reference Sequence XM_002277708.3) and *VvPaO* (pheophorbide *a* oxygenase, NCBI Reference Sequence XM_002274174.3). The housekeeping gene used was *VvTCPB* (T-complex protein 1 subunit beta, Gene ID 100257518) (González-Agüero et al., 2013). The abundance of each transcript was analyzed by real-time PCR with a LightCycler Real-

Fig. 1. Visual color scale for cluster coverage in cv. Crimson Seedless. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Time PCR System (Roche Diagnostics, Mannheim, Germany), using SYBR Green™ as a fluorescent dye to measure the amplified DNA products derived from RNA. Primers were designed using the Primer Premier 5.0 software package (Premier Biosoft International, Palo Alto, CA) and were synthesized by IDT (Integrated DNA Technologies). The primer sequences used in this study are listed in Table S1. The qPCR was performed on four biological replicates for each sample as described by González-Agüero et al. (2008). Relative expression levels were determined using the comparative Ct method of Pfaffl (2001), which was designed to measure the accumulation of mRNA relative to that of a constitutively expressed reference gene.

2.8. Experimental design and statistical analysis

A fully randomized experimental design was used. The data were subjected to statistical analyses of variance, and the means were separated by a least significant difference (LSD) test at 5% significance using the Statgraphics Centurion Plus 5 software package (Manugistics Inc., Rockville, USA).

2.9. Multivariate statistical analysis (principal component analyses (PCA))

To determine the relationship between color development and anthocyanin metabolism in berries treated with ABA and/or sucrose, a principal component analysis (PCA) was performed using InfoStat (version 2015, Universidad Nacional de Cordoba, Argentina). This analysis has two main functions: (1) to indicate relationships among groups of variables and (2) to indicate relationships between objects. PCA proceeds by searching for linear combinations of variables that account for the maximum possible variation of the data and is used to facilitate interpretation of the data based on data visualization (Contreras and Beaudry, 2013). The resulting data matrix contained 18 samples (3 samples per treatment for six treatments) using the same 18 variables for each time point. The variables studied were concentrations of five different types of anthocyanins (Pn, Mv, Cy, Dp and Pt); maturity parameters such as TSS, TA, TSS/TA, color coverage of cluster (CC) and CIRG; and expression of the phenylpropanoid pathway genes (*VvPAL*, *VvCHS*, *VvCHI*, *VvMYBA1* and *VvUFGT*) and the chlorophyll degradation genes (*VvCFL2b*, *VvPaO* and *VvRCCR*). The relation between the anthocyanins, maturity parameters, and expression of genes were estimated considering the Pearson correlation coefficient ($p < 0,05$) using the statistical software InfoStat (Version 2015, Universidad Nacional de Córdoba, Argentina).

3. Results and discussion

3.1. Maturity parameters

To measure maturity attributes, clusters from all treatments were harvested when 15% of the bunches from the control treatment reached class 5 (commercial harvest) according to the color visual scale (Fig. 1).

Total soluble solids showed an increase between *véraison* and harvest for all treatments studied (Fig. 2A). Concomitant with the TSS increase, a reduction in TA was observed during berry development, declining significantly from *véraison* to harvest (Fig. 2B). Moreover, soluble solids, TA and TSS/TA ratio did not show differences among ABA and/or sucrose treatments at any sampling date. These results coincide with those of other authors who have reported the effect of ABA treatments in 'Crimson Seedless' for soluble solids, TA and TSS/TA ratio (Cantín et al., 2007; Peppi et al., 2007; Lurie et al., 2009; Ferrara et al., 2013, 2015). All of these previous studies found that the application of ABA had little or no effect on these maturity parameters. The same results have been found in other cultivars such as 'Cabernet Sauvignon', 'Flame Seedless' and 'Red Globe' (Jeong et al., 2004; Peppi et al., 2006, 2007). Likewise, after sucrose treatments Ferrara et al. (2015) did not observe differences in soluble solids accumulation or acid degradation.

In regard to berry firmness, moderate softening began at *véraison*, with a significant decrease in firmness from 26 DAV, reaching a minimum level of 320.7 g/mm for ABA400 + sucrose and a maximum of 326.4 g/mm for ABA400 at harvest (Fig. 2C). Despite the decrease in firmness, these values are still considered optimal for a commercial harvest. The treatments had no significant effect on berry firmness at any sampling date, and there were no significant differences among the treatments. Other studies on this matter found differing results. Cantín et al. (2007) and Ferrara et al. (2013, 2015) observed that ABA treatments did not affect berry softening. In contrast, Peppi et al. (2007) observed that ABA treatments significantly reduced berry firmness and concluded that the time of application was relevant. They observed a greater effect on softening when ABA was applied at *véraison* rather than 4 weeks later. Likewise, Lurie et al. (2009) reported lower berry firmness in all ABA treatments compared to the control.

3.2. Color development and color quality

As expected, the colors of bunches increased between *véraison* and harvest in all treatments (Cantín et al., 2007; Fernández-López et al., 1998) (Fig. 3). Treatment with ABA and/or sucrose had a significant effect on color development compared to the control. ABA and/or sucrose treated cluster showed more advanced color development from the application date (13 DAV) until harvest (63 DAV). The ABA treatments showed significant differences from 26 DAV, and sucrose from 35 DAV, relative to the control. At harvest (63 DAV), clusters within ABA and/or sucrose treatments were in class 4 or 5 on the visual scale, whereas the ones in control plants were between 3 and 4. These results suggest that a commercial harvest may proceed earlier in ABA and/or sucrose-treated clusters. For instance, the clusters treated with ABA400 reached color stage 5 by 37 days earlier (26 DAV) than untreated grapes, which were in color stage 3 at that time; and sucrose-treated clusters reached color stage 5 15 days earlier than the control (Table S2). Therefore, at harvest, clusters treated with ABA and/or sucrose showed a greater percentage of fruit in full color with commercial potential, i.e., 80% of bunches treated with ABA400 and 38.1% of the sucrose-treated bunches were in class 5 of the visual color scale, respectively, while only 16.2% of the untreated fruit were in class 5 (Table S2). Similar results were found by Cantín et al. (2007) in 'Crimson Seedless', where the percentage of ABA and ethephon-treated packed grapes at harvest were double the amount of the untreated packed grapes, ~80% and 40%, respectively. Therefore, they concluded that grapes treated with ABA at 300 ppm were harvestable ~30 days earlier than untreated grapes and 10 days earlier than grapes treated with ethephon. Ferrara et al. (2013) applied 400 ppm of ABA to 'Crimson Seedless' twice during post-*véraison* and observed that treated grapes colored more quickly, with the fruit being ready for harvest 15 days earlier than the untreated control. Ferrara et al. (2015) reported that 'Crimson Seedless' bunches treated with ABA400 and/or sucrose were harvested in a reduced period of time (less than 2 weeks), whereas control bunches took over a month to complete harvest. Interestingly, they also observed that the skin color of the untreated grapes was lighter and less attractive than that of treated grapes. Overall, all these authors (Cantín et al., 2007; Ferrara et al., 2013, 2015) concluded that these plant growth regulators (ABA, sucrose and ethephon)

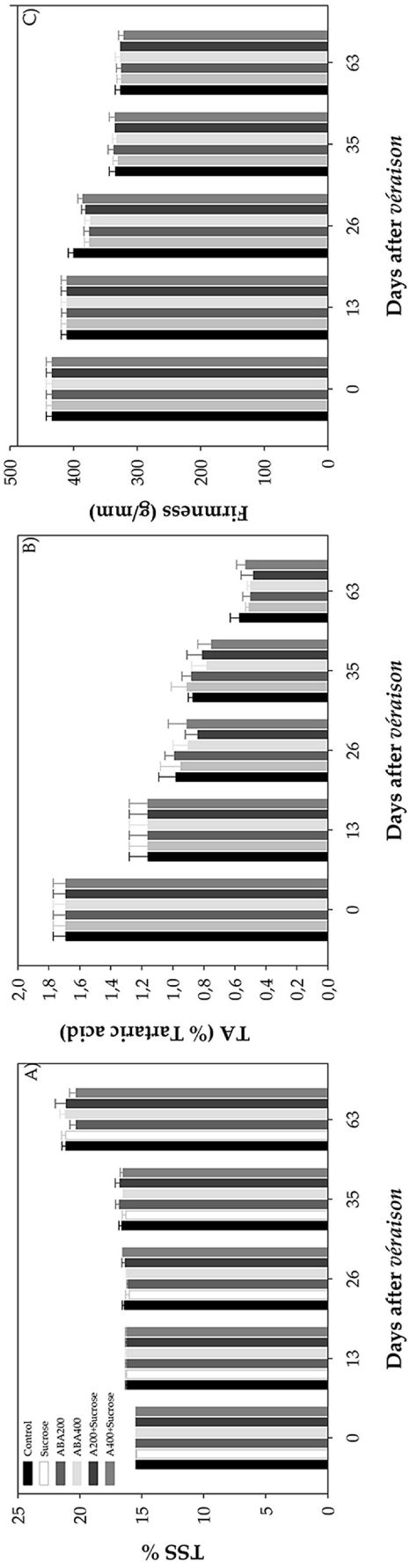

Fig. 2. Maturity parameters of table grape cv. Crimson Seedless from *véraison* (0 DAV) to harvest (63 DAV). A) Total soluble solids (TSS), B) Titratable acidity (TA), and C) Firmness. Bars represent the means of four replicates ± SE.

increased the percentage of harvestable fruit at a certain time period by improving the proportion of fruit that was well-colored. Berry color followed the same pattern of color coverage during development as was observed in the clusters (Fig. S2).

Color quality parameters were measured with a tristimulus colorimeter (CIELAB coordinates). The L^*, a^*, b^*, C^* and h° values for all treatments are shown in Table 1. During berry development, L^* values decreased consistently in all treatments from *véraison* to harvest. Lightness decreased to a greater extent in fruit treated with ABA than in the sucrose and control treatments. After treatments, L^* sharply decreased in fruit treated with ABA from 40.9 (13 DAV) to 33.1–34.8 at 26 DAV and 28.4–30.2 at harvest (63 DAV). On the other hand, fruits subjected to sucrose treatment had a slight decrease in luminosity at 26 and 35 DAV, to reach levels near those of ABA-treated fruits at harvest. The untreated fruit had significantly higher L^* values than fruit treated with ABA and/or sucrose. In general, there were no differences among ABA treatments, but there were significant differences among ABA, sucrose and control treatments (Table 1).

Fruit treated with ABA and/or sucrose had lower C^* values than non-treated fruits, suggesting that the treated fruit had slightly less pure color than the non-treated fruits. However, this slight effect was not perceived by the unaided eye (Cantín et al., 2007; Ferrara et al., 2013).

The parameter a^* increased during berry development. At *véraison*, a^* had negative values, corresponding to green color; this then transitioned to positive a^* values or red color at harvest (Table 1). On the other hand, b^* values decreased during development, to a darker violet color. Interestingly, b^* followed the same pattern as L^* in all treatments, i.e., control and sucrose treatments were significantly higher than ABA treatments, and there were no differences among ABA treatments.

The hue angle decreased during berry development in all treatments, dropping from a value of 112.66 at *véraison* (green color) to a range from 17.4 to 30.2 (red color) at harvest. A dramatic decrease was observed in the ABA400 treatment, which decreased to a value of 39.5 at 26 DAV, whereas the remaining treatments showed similar lower hue values at 35 DAV. ABA400 treatment likely accelerated the pigmentation process by ~10 days; this effect

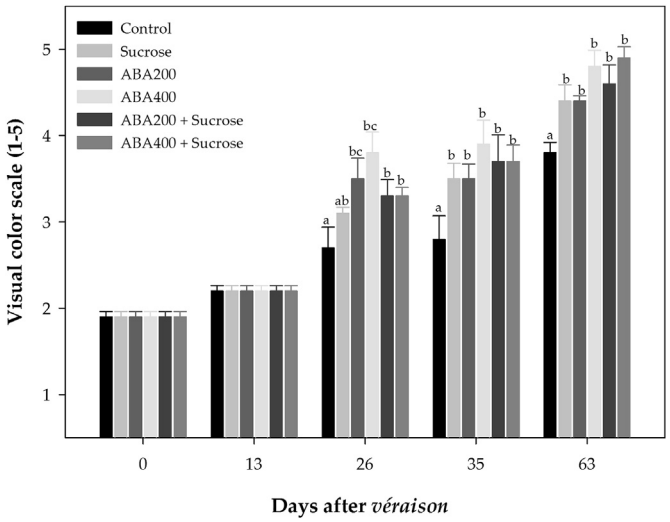

Fig. 3. Color development of clusters in table grape cv. Crimson Seedless from *véraison* (0 DAV) to harvest (63 DAV). Bars represent the means of four replicates ± SE. Different letters indicates significant differences among treatments within each sampling time by LSD analysis ($p < 0.05$).

was also observed in the color coverage of clusters and berries mentioned above (Fig. 3; Fig. S2).

Based on the CIRG index, untreated berries were classified as pink (2 < CIRG<4) and the berries of other treatments as red-violet (4 < CIRG<5) at harvest (Table 1) (Cantín et al., 2007; Ferrara et al., 2013, 2015). The same behavior as seen for the hue angle was observed in ABA400-treated fruit since it reached pink color earlier than the other treatments, at 26 DAV. This treatment also showed darker color at harvest, but with no significant differences when compared to the other treatments.

The behaviors of the CIRG index and color parameters L^*, C^* and hue were similar to those observed in previous studies in cv. Crimson Seedless (Cantín et al., 2007; Peppi et al., 2007; Lurie et al., 2009; Ferrara et al., 2013, 2015). In general, ABA and/or sucrose treatments had a significant effect on color development, accelerating the pigmentation process of the grapes. A plausible cause for this change in color by these plant growth regulators may be the induction of increased expression of some transcription factors and structural genes in the phenylpropanoid pathway, leading to anthocyanin accumulation (Peppi et al., 2008; Gagné et al., 2011).

3.3. Anthocyanins

Total anthocyanin accumulation increased from *véraison* to harvest in all treatments (Fig. 4A). The effect of exogenous application of ABA was significantly different from the effects of sucrose and control treatments; that is, ABA treatments significantly increased the levels of anthocyanins in grape skin (Peppi et al., 2007; Ferrara et al., 2015). Therefore, the anthocyanin levels of sucrose-treated grapes were lower than those of grapes treated with ABA and higher than those of the untreated control. The ABA400 treatment showed the highest accumulation of total anthocyanins, confirming our previous data on color coverage, CIRG index and hue angle. These findings coincide with the observations of Ferrara et al. (2015). At harvest, the ABA400 treatment in total anthocyanin content was 5.6-fold and 3.1-fold higher than the control and sucrose treatments, respectively (Table S3).

Twelve different anthocyanins were identified and quantified: the 3-O-monoglucosides of peonidin (PnG), cyanidin (CyG), malvidin (MvG), delphinidin (DpG), and petunidin (PtG), and their acetylated (PnAG, CyAG, MvAG, and DpAC) and coumaroylated

Table 1
Effect of ABA and/or sucrose treatments in cv. Crimson Seedless on quality color of berries from *véraison* to harvest.

Treatments			L^*		
	0 DAV	13 DAV	26 DAV	35 DAV	63 DAV
Control	43.9 ± 0.14	40.9 ± 0.85	38.8 ± 0.23a	38.6 ± 0.47a	32.7 ± 0.29a
Sucrose			37.9 ± 1.49 ab	36.4 ± 0.79b	30.7 ± 0.28b
ABA200			34.8 ± 0.78bc	32.5 ± 0.74c	28.4 ± 1.16c
ABA400			33.4 ± 1.41c	32.8 ± 0.30c	28.8 ± 0.49bc
ABA200 + Sucrose			33.1 ± 0.81c	31.9 ± 0.41c	30.2 ± 0.52bc
ABA400 + Sucrose			34.6 ± 1.11c	32.9 ± 0.54c	30.1 ± 0.85bc
			C^*		
Control	10.7 ± 0.76	9.9 ± 0.45	9.61 ± 0.44 ns	9.14 ± 0.15 ns	8.69 ± 0.21a
Sucrose			9.03 ± 1.09 ns	8.06 ± 0.84 ns	6.91 ± 0.42b
ABA200			7.10 ± 0.52 ns	7.07 ± 1.00 ns	6.11 ± 0.37bc
ABA400			6.92 ± 0.51 ns	6.88 ± 1.01 ns	5.65 ± 0.66cd
ABA200 + Sucrose			7.84 ± 0.86 ns	7.21 ± 0.65 ns	5.40 ± 0.17cd
ABA400 + Sucrose			7.02 ± 0.49 ns	5.91 ± 0.50 ns	5.25 ± 0.53d
			a^*		
Control	−4.4 ± 0.79	−2.2 ± 0.37	0.5 ± 1.84 ns	3.9 ± 0.29c	8.1 ± 0.56 ns
Sucrose			1.9 ± 1.41 ns	2.7 ± 0.38d	8.4 ± 0.94 ns
ABA200			4.0 ± 0.39 ns	5.9 ± 0.42a	6.2 ± 0.66 ns
ABA400			4.7 ± 0.27 ns	5.4 ± 0.65 ab	6.4 ± 0.66 ns
ABA200 + Sucrose			4.0 ± 0.24 ns	4.7 ± 0.18abc	7.0 ± 0.45 ns
ABA400 + Sucrose			3.4 ± 0.27 ns	4.5 ± 0.22bc	6.4 ± 0.38 ns
			b^*		
Control	9.6 ± 0.53	8.5 ± 0.44	7.8 ± 0.49a	7.8 ± 0.38a	4.7 ± 0.33a
Sucrose			7.3 ± 1.26a	5.6 ± 0.65b	2.9 ± 0.61b
ABA200			4.3 ± 0.71b	3.2 ± 0.75c	2.7 ± 0.79b
ABA400			3.9 ± 0.99b	2.3 ± 0.29c	2.3 ± 0.60b
ABA200 + Sucrose			3.9 ± 0.65b	3.5 ± 0.69c	2.7 ± 0.33b
ABA400 + Sucrose			3.8 ± 0.83b	2.8 ± 0.49c	2.5 ± 0.30b
			h°		
Control	112.7 ± 3.43	97.8 ± 3.48	100.8 ± 10.67a	62.4 ± 2.62a	30.2 ± 2.69a
Sucrose			105.6 ± 16.10a	66.9 ± 12.90a	17.4 ± 2.43b
ABA200			103.1 ± 7.43a	32.0 ± 3.57bc	20.5 ± 4.45b
ABA400			39.5 ± 5.57b	23.3 ± 2.37c	20.3 ± 2.52b
ABA200 + Sucrose			108.1 ± 2.03a	37.9 ± 3.35bc	19.7 ± 1.66b
ABA400 + Sucrose			101.9 ± 2.04a	46.8 ± 8.61 ab	18.2 ± 1.09b
			CIRG		
Control	1.30 ± 0.09	1.59 ± 0.09	1.78 ± 0.21b	2.40 ± 0.03b	3.68 ± 0.02b
Sucrose			1.85 ± 0.04b	2.78 ± 0.28b	4.26 ± 0.27a
ABA200			1.98 ± 0.08b	3.54 ± 0.11 ab	4.53 ± 0.33a
ABA400			3.58 ± 0.33a	3.89 ± 0.04a	4.51 ± 0.05a
ABA200 + Sucrose			1.72 ± 0.07b	3.49 ± 0.09 ab	4.46 ± 0.02a

Data are represented as the means from four replicates ± SE. Different letters within a column indicate significant differences by LSD analysis ($p < 0.05$). ns = not significant.

derivative forms (PnAC, CyAC, and MvAC) (Fig. S3 and Table S3).

The di-hydroxylated anthocyanins, peonidin and cyanidin, paralleled the pattern shown by the total anthocyanin content (Fig. 4B). This is explained by the PnG form, which represents the 68.9% of the total anthocyanin content; as CyG represents 11.2%, these anthocyanins accounted for the 80% of total anthocyanin content. Other studies have also reported PnG as the predominant form in cv. 'Crimson Seedless' (Cantos et al., 2002; Singh Brar et al., 2008; Ferrara et al., 2015). For instance, Cantos et al. (2002) reported relative contents of 45.2% for PnG and 6.6% for CyG; and Ferrara et al. (2015) found that PnG composed approximately 85% and CyG approximately 4.7% of total anthocyanins.

The tri-hydroxylated anthocyanins, malvidin (15.5%), delphinidin (2.8%) and petunidin (1.6%), also showed the same behavior discussed above, but in lesser amounts (Fig. 4C). The ABA-treated grapes showed increased levels of the tri-hydroxylated anthocyanins. Unlike the total anthocyanin content and the di-hydroxylated anthocyanin content, sucrose and control treatments had no effect on the tri-hydroxylated anthocyanins.

3.4. Chlorophyll content

Green color loss has been associated with chlorophyll breakdown and is a visual sign of fruit ripening (Barry et al., 2008). In our study, total chlorophyll content from *véraison* to harvest remained constant in the control treatment, likely due to a limited chlorophyll degradation rate (Fig. 5A). This result differs from previous studies on chlorophyll content in other grape varieties such as 'Merlot', 'Chardonnay' and 'Cabernet Sauvignon', in which chlorophyll content decreases during development and fruit ripening (Fougère-Rifot et al., 1995; Gény et al., 2005). After ABA and/or sucrose applications at 13 DAV, a sharp increase in chlorophyll content was observed in all treatments; this decreased again from 35 DAV until harvest (Fig. 5A). Gény et al. (2005) reported a significant decrease in chlorophyll content in 'Merlot' berries treated with ABA, contrary to what was observed in our study.

The chlorophyll *a* content remained relatively unchanged in all treatments during grape development (Fig. 5B). On the other hand, the same burst shown in the total chlorophyll content (Fig. 5A) was also observed in chlorophyll *b* content (Fig. 5C). These results differ from those reported by Gény et al. (2005) in 'Merlot', where the contents of chlorophyll *a* and chlorophyll *b* decreased during development. Studies on the effect of ABA on chlorophyll metabolites in other fruits such as litchi have shown that ABA treatments slow or have no effect on the chlorophyll degradation rate, as opposed to what has been observed with other hormones such as ethylene, which accelerates the degradation of chlorophyll (Lai et al., 2015; Wang et al., 2007).

3.5. Expression of phenylpropanoid pathway genes

The genes upstream in the flavonoid pathway, *VvPAL*, *VvCHS* and *VvCHI*, showed low levels of expression during berry development (Fig. 6). Gene expression of *VvPAL* for all treatments, except for the control, increased after the application date (13 DAV) (Fig. 6A). The *VvCHS* expression profile showed a peak at 13 DAV (except in the ABA400 + sucrose treatment) and then declined in all treatments (Fig. 6B). For *VvCHI*, relative gene expression declined throughout development, except in ABA400 + sucrose and ABA200 + sucrose, which had peaks at 26 and 35 DAV, respectively (Fig. 6C). Interestingly, ABA400 + sucrose-treated fruits had a peak of expression of all genes at 26 DAV, with a 2-fold increase in *VvPAL* relative to *véraison*, a nearly 3-fold increase in *VvCHS* and a 1.45-fold increase in *VvCHI*. Other studies in 'Cabernet Sauvignon' have shown that abscisic acid induced the upregulation of some structural genes of

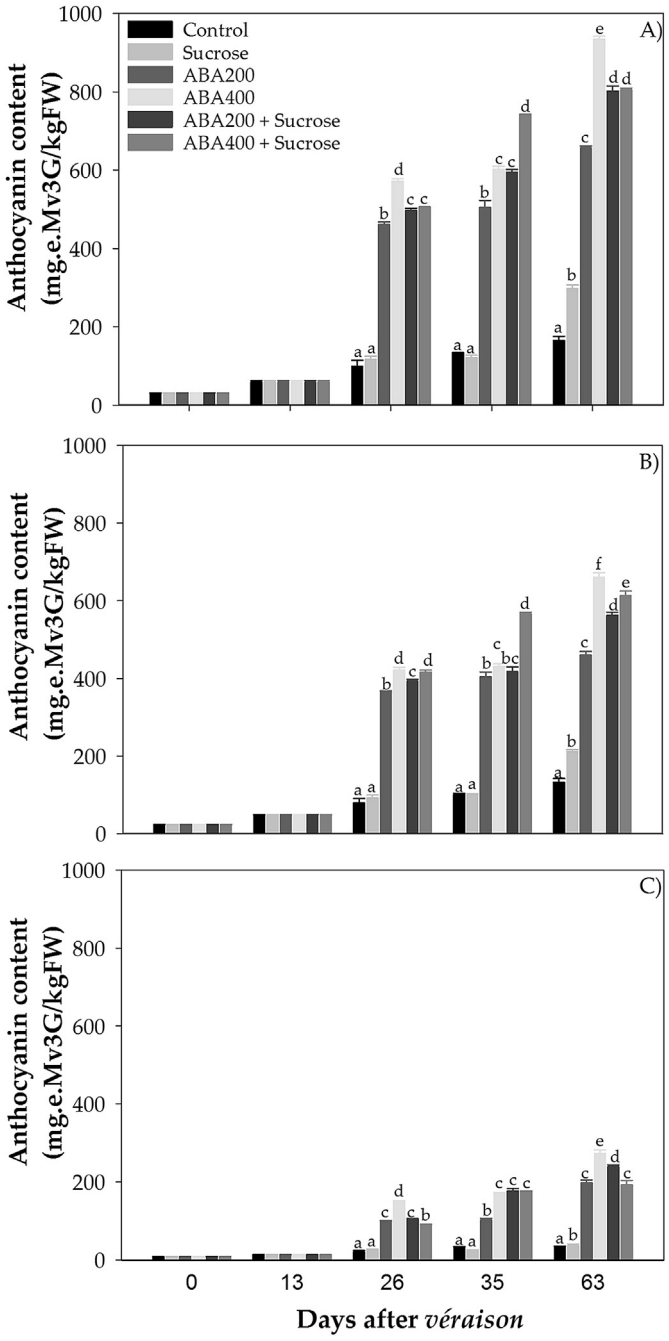

Fig. 4. Anthocyanin contents in cv. Crimson Seedless from *véraison* (0 DAV) to harvest (63 DAV). A) Total anthocyanins, B) Di-hydroxylated anthocyanins, C) Tri-hydroxylated anthocyanins. Bars represent the means of four replicates ± SE. Different letters indicates significant differences among treatments within each sampling time by LSD analysis ($p < 0.05$).

the phenylpropanoid pathway: *VvPAL*, *VvCHS* and *VvCHI*. These genes were rapidly and significantly induced after ABA treatment; modulation of subsequent expression patterns differed for each gene (Jeong et al., 2004; Koyama et al., 2010; Gagné et al., 2010, 2011). Ban et al. (2003) reported similar results in 'Kyoho' grape berries; ABA increased the expression of *VvPAL*, *VvCHS* and *VvCHI* genes at 7 days after treatment. Additionally, in cell suspensions from *Vitis vinifera* cv. Barbera berries treated with sucrose, increased expression of *VvPAL*, *VvCHS* and *VvCHI* genes was shown (Ferri et al., 2011). Our results differ from these studies in which a

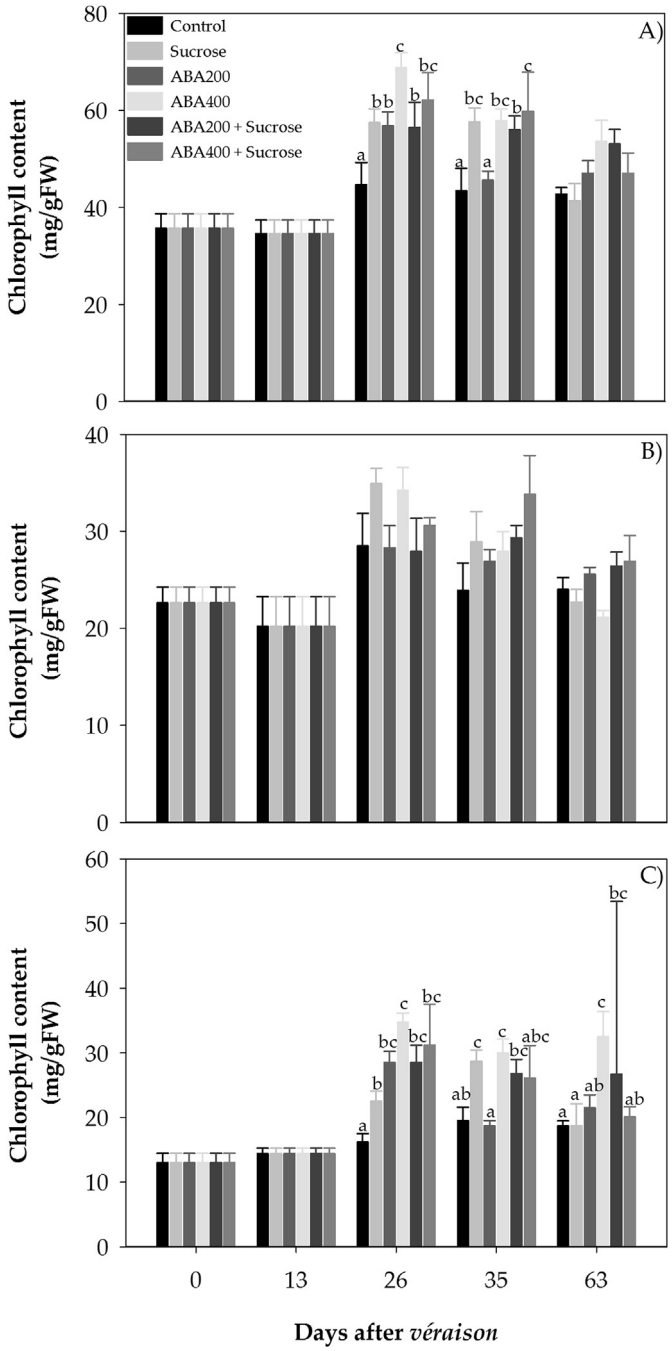

Fig. 5. Chlorophyll contents in cv. Crimson Seedless from *véraison* (0 DAV) to harvest (63 DAV). A) Total Chlorophyll. B) Chlorophyll *a*. C) Chlorophyll *b*. Bars represent the means of four replicates ± SE. Different letters indicates significant differences among treatments within each sampling time by LSD analysis ($p < 0.05$).

peak of expression was observed after ABA and/or sucrose application. In our work, only *VvPAL,VvCHS* and *VvCHI* under ABA400 + sucrose treatment showed pattern similar to that described in the literature. It is likely that *VvCHS* and *VvCHI* did not show this behavior because the treatments in our study were applied 2 weeks post-*véraison*, when the induction of some genes had already been triggered; whereas in the other studies the treatments were applied at *véraison*.

For the downstream genes, *VvMYBA1* and *VvUFGT*, the expression levels were higher than those of the genes previously discussed (Fig. 6D and E). Coincidently with the color parameter patterns discussed above, *VvMYBA1* and *VvUFGT* showed increased expression at 26 DAV and expression then declined towards harvest. As was the case for the other genes (*VvPAL*, *VvCHS* and *VvCHI*), ABA400 + sucrose induced the expression of *VvMYBA1* at 26 DAV, with a 5-fold increase relative to *véraison*. However, for *VvUFGT*, ABA200-treated fruits showed the highest expression, followed by ABA400 + sucrose. Other studies have also reported that transcription of the regulator *VvMYBA1* increased after ABA treatment in 'Cabernet Sauvignon' grapes (Jeong et al., 2004; Gagné et al., 2011). Additionally, exogenous ABA treatment transiently induced the expression of *UFGT* in different grape cultivars such as 'Cabernet Sauvignon', 'Kyoho' and 'Crimson Seedless' (Jeong et al., 2004; Koyama et al., 2010; Gagné et al., 2010, 2011; Ban et al., 2003; Peppi et al., 2008). Sucrose has also been shown to induce expression of genes of the phenylpropanoid pathway; Ferri et al. (2011) showed that sucrose treatments in cell suspension from 'Barbera' berries induced *UFGT* expression.

Many ABA-inducible genes contain a conserved ABA-responsive, cis-acting elements, known as ABA-responsive element (ABRE; core-sequence: ACGTGG/TG) and coupling-element (CE; core-sequence: CACC) in their promoter regions (Azuma et al., 2009; Chervin et al., 2009). These ABA-inducible genes usually possess one or more ABRE-like and CE-like sequences in their promoter regions. For example, in the promoter region of *VvMYBA1* in cv. Benitaka three ABRE-like and CE-like sequences were identified (Azuma et al., 2009). In Shiraz cultivar, 14 ABRE-like sequences have been described in the *ufgt* promoter region. The Shiraz *ufgt* promoter sequence have 97–99% homology to the grapevine *ufgt* promoter isolated from Kyoho, Italia, Ruby Oku, Muscat of Alexandria, and Flame Muscat cultivars, and 95% to Cabernet Sauvignon (Chervin et al., 2009). Considering the high level of homology among these grapes, it is likely that the promoter region in 'Crimson Seedless' cultivar also contains these elements; although this has to be further proven.

Sucrose has also been reported to induce gene expression by sucrose responsive elements (SURE) and W box components contained in their promoter regions (Jia et al., 2017). For example, in the promoter region of *VvUFGT* in cv. Shiraz five SURE-like sequences were identified (Chervin et al., 2009). Then, ABRE-like and SURE-like sequences can be present at the same time in the promoter of genes, suggesting that sucrose and ABA could regulate together the expression of some genes. Jia et al. (2017) proposed a sucrose-ABA signal transduction system that might be regulating the expression of functional genes during plant growth and development. Furthermore, epigenetic mechanisms also play important roles in anthocyanin biosynthesis (Wang et al., 2013; Zabala and Vodkin, 2014).

3.6. Expression of chlorophyll degradation pathway genes

A sharp decline was observed in the three genes of the chlorophyll degradation pathway, *VvCFL2*, *VvPaO* and *VvRCCR*, at 26 DAV, but expression increased at 35 DAV (Fig. 6F, G and H). This decrease in expression level is consistent with the peak observed in the chlorophyll content after the application of treatments (26 DAV) (Fig. 5). It has been well documented that ABA regulates numerous responses to abiotic and biotic stresses in plants (Zhang et al., 2009b; Gagné et al., 2011). Further, it is known that genes involved in chlorophyll catabolism are upregulated after biotic and abiotic stresses (Zimmermann et al., 2004). Interestingly, in our study the chlorophyll catabolic genes were downregulated in response to ABA treatment.

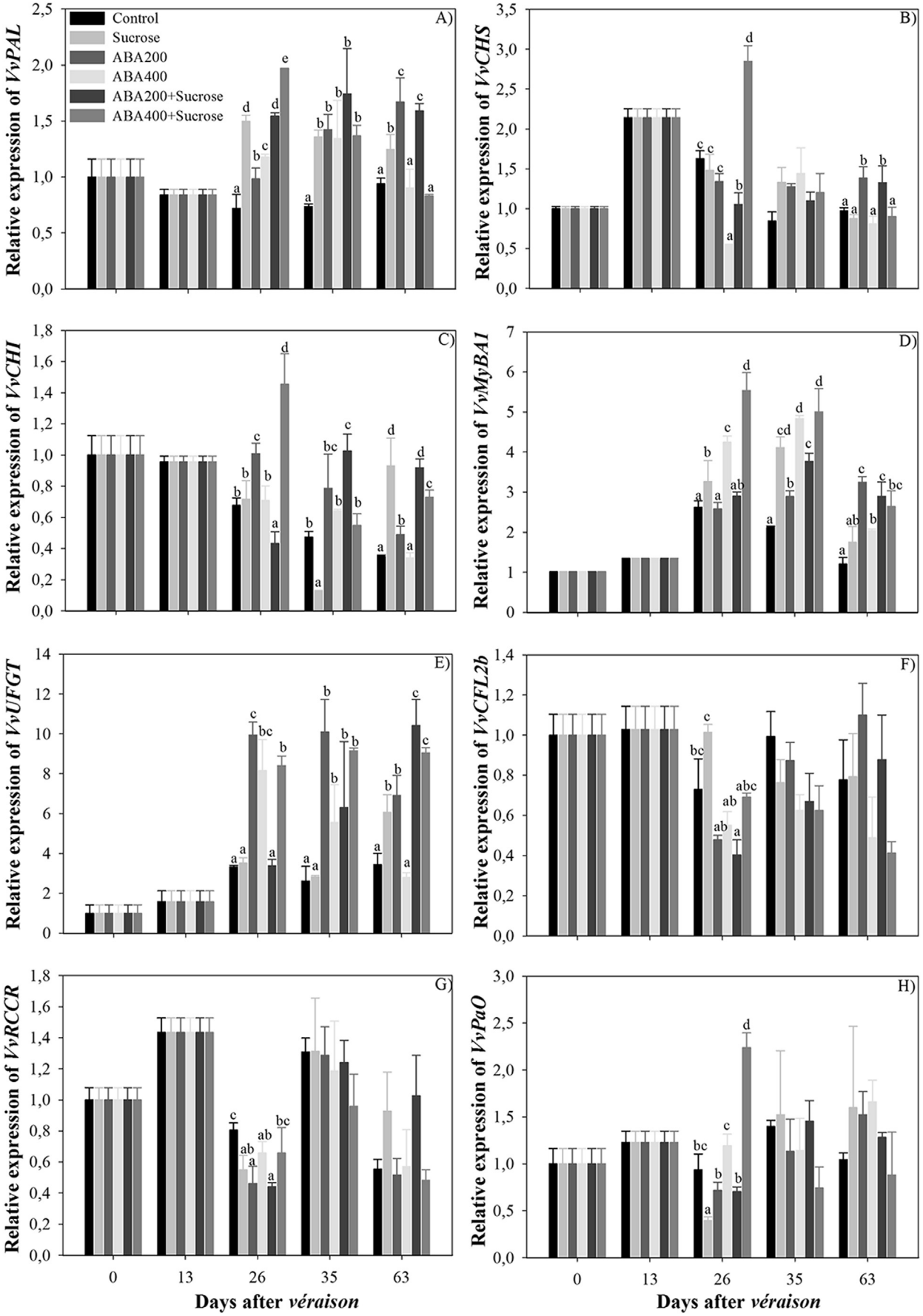

Fig. 6. Relative expression analyses of genes from the phenylpropanoid biosynthetic and chlorophyll degradation pathways. A) *VvPAL*, B) *VvCHS*, C) *VvCHI*, D) *VvMybA1* E) *VvUFGT*, F) *VvCFL2b*, G) *VvRCCR*, H) *VvPaO*. Expression was normalized with the house-keeping gene *VvTCPB* and relative to *véraison*. Bars represent the means of four replicates ± SE. Different letters indicates significant differences among treatments within each sampling time by LSD analysis ($p < 0.05$).

3.7. Multivariate statistical analysis (principal component analyses (PCA))

For 26 DAV, 63.3% of the variability of the data was accounted for by two principal components (PC). PC1 and PC2 explained 41.0 and 22.3% of the variability of the data, respectively (Fig. 7). Within PC1, the variables that exerted more weight on the analysis were the anthocyanin forms Pn, Mv, Cy, Pt and Dp. In the case of PC2, the genes of chlorophyll degradation (*VvCFL2b*, *VvRCCR* and *VvPaO*) and TSS were the most important factors. Significant and positive correlations were observed between color variables and anthocyanin contents according to the data matrix (data not shown): CC and Pn ($r = 0.63$, $p = 0.0054$), Mv ($r = 0.76$, $p = 0.0003$), Pt ($r = 0.67$, $p = 0.0023$) and CIRG ($r = 0.60$, $p = 0.0078$). Similarly, CIRG positively correlated with anthocyanins Pn ($r = 0.53$, $p = 0.0222$), Mv ($r = 0.75$, $p = 0.0003$) and Pt ($r = 0.67$, $p = 0.0023$). In contrast, the predominant anthocyanin Pn was significantly and negatively correlated with *VvCFL2b* ($r = -0.68$, $p = 0.0018$) (Fig. 7).

The treatments were distributed along PC1 and clustered in four different groups. Interestingly, the control and sucrose treatment clustered together with negative values in the PC1, whereas ABA and ABA + sucrose treatments had positive values in PC1. Notably, ABA400 + sucrose grouped close to the transcription factor *VvMYBA1* and to most of the structural phenylpropanoid genes, whereas ABA400 treatment was clustered close to anthocyanins and the *VvPAL* gene. This may suggest a strong synergistic effect between ABA400 and sucrose in the induction of important genes.

This synergistic effect of ABA400 + sucrose that was observed in some the phenylpropanoid pathway genes was not observed at the metabolite level at 26 DAV (Fig. 4). Therefore, as seen in Fig. S3, the ABA400 and ABA400 + sucrose treatments showed no differences in their anthocyanin profiles (Pn, Mv, Pt, Cy and Dp).

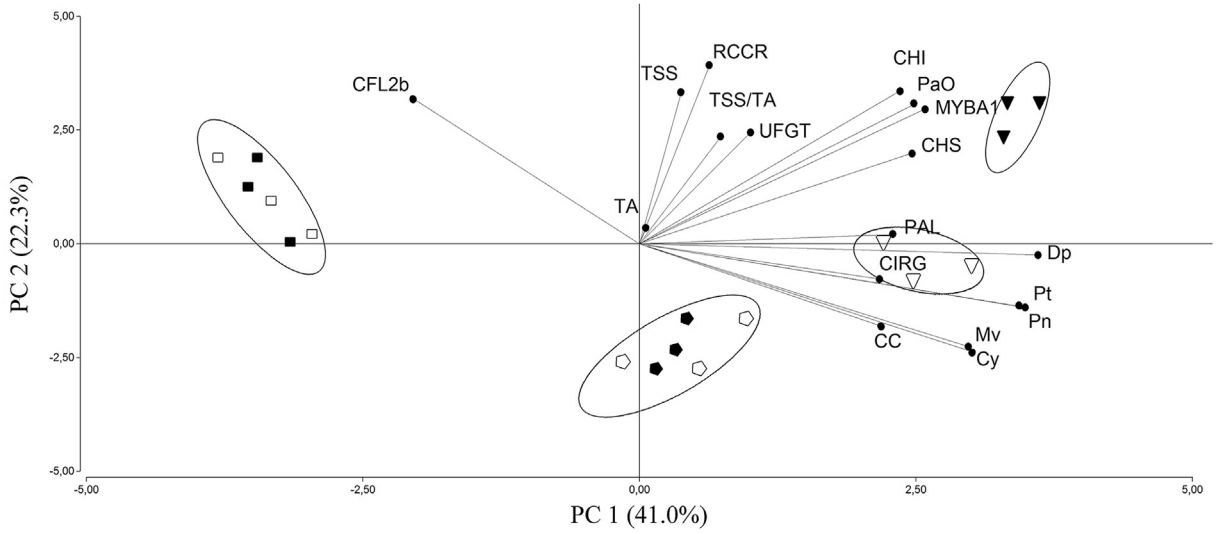

Fig. 7. Principal component analysis (PCA) in CS at 26 DAV. Biplot (loading variables and samples scores) of PC1 vs PC2 are presented. Open square symbols correspond to untreated fruit, closed square symbols to fruit treated with sucrose, open pentagon symbols to fruit treated with ABA200, closed pentagon symbols to fruit treated with ABA200 + sucrose, open triangle symbols to fruit treated with ABA400 and closed triangle symbols to fruits treated with ABA400 + sucrose.

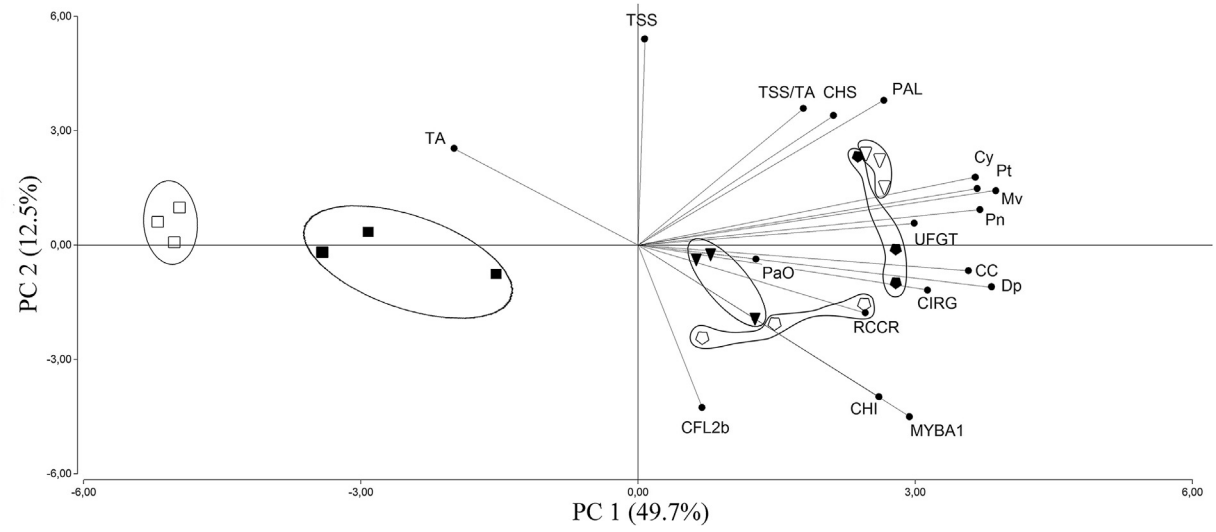

Fig. 8. Principal component analysis (PCA) in CS at harvest. Biplot (loading variables and samples scores) of PC1 vs PC2 are presented. Open square symbols correspond to untreated fruit, closed square symbols to fruit treated with sucrose, open pentagon symbols to fruit treated with ABA200, closed pentagon symbols to fruit treated with ABA200 + sucrose, open triangle symbols to fruit treated with ABA400 and closed triangle symbols to fruits treated with ABA400 + sucrose.

At harvest, PC1 contained 49.7% of the total variation whereas PC2 contained 12.5% (Fig. 8). As was observed at 26 DAV, anthocyanins and CC weighted most heavily in PC1. For PC2, the most influential variables were TSS, *VvPAL*, *VvCHI*, *VvMYBA1* and *VvCFL2b*. The color coverage of the cluster was positively and significantly related to *VvMYBA1* ($r = 0.73$, $p = 0.006$), *VvUFGT* ($r = 0.55$, $p = 0.0182$) and the anthocyanins Pn ($r = 0.85$, $p < 0.0001$) and Dp ($r = 0.86$, $p < 0.0001$) (data not shown). On the other hand, TA was negatively and significantly correlated to CC ($r = -0.48$, $p = 0.0441$) and CIRG ($r = -0.55$, $p = 0.0171$) (Fig. 8).

During harvest, the treatments with ABA (ABA200, ABA400 and in combination with sucrose) were clustered with positive values in PC1 with variables such as CC, CIRG, anthocyanins, and expressed genes. In the case of sucrose, this treatment was related to intermediate negative values, as opposed to the control that was related to more negative values. Unlike the results at 26 DAV, all treatments separated along component 1, and showed a disperse distribution among the variables.

4. Conclusions

We demonstrated that the applications of ABA and/or sucrose had a significant effect on the development of color, being able to advance the harvest by 37 and 15 days, respectively. Interestingly, this increase in color did not affect other important quality attributes in grapes such as firmness, soluble solids and acidity, unlike the effects observed with other growth regulators such as ethephon. ABA400 and ABA400 + sucrose were the most effective treatments, accelerating the pigmentation process by significantly increasing anthocyanin levels. Sucrose treatment also led to increased anthocyanin content, but to a lesser extent than ABA treatment. The increase in anthocyanins was due to an increase in the expression of key genes in the anthocyanin biosynthesis pathway, such as *VvMYBA1* and *VvUFGT*. The use of ABA + sucrose improved fruit quality by increasing the intensity and development of color; these treatments would therefore promote earlier harvests, lower costs by reducing the number of harvests, and decrease losses due to climatic conditions. Due to the need for an economic alternative for table grape production, further studies of sucrose applications (dose and application time) are needed.

Author contributions

All the authors contribute to the study supporting this manuscript. The conception and design of experiments was performed by Daniela Olivares with the collaboration of Bruno G. Defilippi and Julio Retamales. Daniela Olivares performed the experiments. Victoria Muñoz performed RNA extraction, cDNA and gene expression of phenylpropanoid pathway. Sebastían Rivera performed Multivariate statistical analysis (PCA). Daniela Olivares, Bruno G. Defilippi, Julio Retamales, Carolina Contreras, Sebastían Rivera and Mauricio González-Agüero interpreted and discussed the results. Daniela Olivares and Carolina Contreras wrote the paper.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This study was fully funded by a FONDECYT grant 3140065 (Conicyt, Chile). Authors thank Dr. Alvaro Peña and Hector Morales from Department of Enology (Food Science) of University of Chile, for technical support on HPLC-DAD analysis.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.plaphy.2017.04.007.

References

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24 (1), 1–14.
Azuma, A., Kobayashi, S., Goto-Yamamoto, N., Shiraishi, M., Mitani, N., Yakushiji, H., Koshita, Y., 2009. Color recovery in berries of grape (*Vitis vinifera* L.) 'Benitaka', a bud sport of 'Italia', is caused by a novel allele at the VvmybA1 locus. Plant Sci. 176 (4), 470–478.
Ban, T., Ishimaru, M., Kobayashi, S., Shiozaki, S., Goto-Yamamoto, N., 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. Biotech. 78, 586–589.
Barry, C.S., McQuinn, R.P., Chung, M.Y., Besuden, A., Giovannoni, J.J., 2008. Amino acid substitutions in homologs of the STAY-GREEN protein are responsible for the green-flesh and chlorophyll retainer mutations of tomato and pepper. Plant Physiol. 147 (1), 179–187.
Bravo, J., 2014. Boletín Frutícola Avance Marzo 2014. Odepa. http://www.odepa.cl/wp-content/files_mf/1398089827BoletinfrutC3ADcola2014_04.pdf.
Boss, P.K., Davies, C., Robinson, S.P., 1996. Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. cv. Shiraz grape berries and the implications for pathway regulation. Plant Physiol. 111 (4), 1059–1066.
Cameron, I., 2001. Crimson Seedless promise WA table grape boon. J. Agric. 42 (1), 24–29.
Cantín, C.M., Fidelibus, M.W., Crisosto, C.H., 2007. Application of abscisic acid (ABA) at veraison advanced red color development and maintained postharvest quality of 'Crimson Seedless' grapes. Postharvest Biol. Technol. 46, 237–241.
Cantos, E., Espián, J.C., Tomás-Barberán, F.A., 2002. Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. J. Agric. Food Chem. 50, 5691–5696.
Carreño, J., Martínez, A., Almela, L., Fernández-López, J.A., 1995. Proposal of an index for the objective evaluation of the colour of red table grapes. Food Res. Int. 28, 373–377.
Carreño, J., Martínez, A., Almela, L., Fernández-López, J.A., 1996. Measuring the color of table grapes. Color Res. Appl. 21 (1), 50–54.
Chervin, C., Tira-Umphon, A., Chatelet, P., Jauneau, A., Boss, P.K., Tesniere, C., 2009. Ethylene and other stimuli affect expression of the UDP glucose-flavonoid 3-*O*-glucosyltransferase in a non-climacteric fruit. Vitis 48 (1), 11–16.
Contreras, C., Beaudry, R., 2013. Lipoxygenase-associated apple volatiles and their relationship with aroma perception during ripening. Postharvest Biol. Technol. 82, 28–38.
Dai, Z.W., Meddar, M., Renaud, C., Merlin, I., Hilbert, G., Delrot, S., Gomès, E., 2014. Long-term in vitro culture of grape berries and its application to assess the effects of sugar supply on anthocyanin accumulation. J. Exp. Bot. 65 (16), 4665–4677.
Del Valle, G., González, A., Báez, R., 2005. Grape anthocyanins (*Vitis vinifera* L.) and their relation to color. Rev. Fitotec. Mex. 28 (4), 359–368.
Dokoozlian, N.K., Luvisi, D.A., Schrader, P.L., and Moriyama, M.M., 1994. Influence of trunk girdle timing and ethephon on the quality of 'Crimson Seedless' table grapes. In: Rantz, J.M. (Ed.), International Symposium on Table Grapes Production Proceedings, June 28–29, Anaheim, CA, pp 237–240.
Dokoozlian, N.K., Luvisi, D.A., Moriyama, M.M., Schrader, P.L., 1995. Cultural practices improve color, size of 'Crimson Seedless' grapes. Calif. Agric. 49, 36–39.
Fernández-López, J.A., Almela, L., Muñoz, J.A., Hidalgo, V., Carreño, J., 1998. Dependence between colour and individual anthocyanin content in ripening grapes. Food Res. Int. 31 (9), 667–672.
Ferrara, G., Mazzeo, A., Matarrese, A.M.S., Pacucci, C., Pacifico, A., Gambacorta, G., Faccia, M., Trani, A., Gallo, V., Cafagna, I., Mastrorilli, P., 2013. Application of abscisic acid (S-ABA) to "Crimson Seedless" grape berries in a Mediterranean climate: effects on color, chemical characteristics, metabolic profile, and S-ABA concentration. J. Plant Growth Regul. 32 (3), 491–505.
Ferrara, G., Mazzeo, A., Matarrese, A.M.S., Pacucci, C., Punzi, R., Faccia, M., Trani, A., Gambacorta, G., 2015. Application of abscisic acid (S-ABA) and sucrose to improve colour, anthocyanin content and antioxidant activity of cv. Crimson Seedless grape berries. Aust. J. Grape Wine Res. 21, 18–29.
Ferri, M., Righetti, L., Tassoni, A., 2011. Increasing sucrose concentrations promote phenylpropanoid biosynthesis in grapevine cell cultures. J. Plant Physiol. 168, 189–195.
Fougère Rifot, M., Park, H.S., Cherrad, M., Darné, G., Bouard, J., 1995. Plastids, starch and chlorophyll in *Vitis vinifera* L. berry during its ontogenesis. OENO One 29 (4), 213–225.
Gagné, S., Lacampagne, S., Gény, L., 2010. Impact of abscisic acid on the metabolism of phenolic compounds in grape skins: modulation of anthocyanin and tannin accumulation throughout growth. Acta Hortic. 877, 1159–1166.
Gagné, S., Cluzet, S., Mérillon, J.M., Gény, L., 2011. ABA initiates anthocyanin production in grape cell cultures. J. Plant Growth Regul. 30, 1–10.
Gambetta, G.A., Matthews, M.A., Shaghasi, T.H., McElrone, A.J., Castellarin, S.D., 2010. Sugar and abscisic acid signaling orthologs are activated at the onset of ripening in grape. Planta 232 (1), 219–234.

Gény, L., Deytieux, C., Donèche, B., 2005. Importance of hormonal profile on the onset of ripening in grape berries of *Vitis vinifera* L. Acta Hortic. 682, 99–106.
González-Agüero, M., Pavez, L., Ibáñez, F., Pacheco, I., Campos-Vargas, R., Meisel, L.A., Orellana, A., Retamales, J., Silva, H., González, M., Cambiazo, V., 2008. Identification of woolliness response genes in peach fruit after post-harvest treatments. J. Exp. Bot. 59 (8), 1973–1986.
González-Agüero, M., García-Rojas, M., Di Genova, A., Correa, J., Maass, A., Orellana, A., Hinrichsen, P., 2013. Identification of two putative reference genes from grapevine suitable for gene expression analysis in berry and related tissues derived from RNA-Seq data. BMC Genom 14, 878.
Gudenschwager, O., González-Agüero, M., Defilippi, B.G., 2012. A general method for high-quality RNA isolation from metabolite-rich fruits. S. Afr. J. Bot. 83, 186–192.
Jensen, F.L., Kissler, J., Peacock, W., Leavitt, G., 1975. Effect of ethephon on color and fruit characteristics of Tokay and Emperor table grapes. Amer. J. Enol. Vitic. 26, 79–81.
Jensen, F.L., Kissler, J., Peacock, W., Leavitt, G., Andris, H., Luvisi, D., 1982. Color and maturity promotion in table grapes with ethephon. Grape and Wine Centennial Symp. Proc. Univ. of California Press, Davis, pp. 118–121.
Jeong, S.T., 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, H., Xie, Z., Wang, C., Shangguan, L., Qian, N., Cui, M., Liu, Z., Zheng, T., Wang, M., Fang, J., 2017. Abscisic acid, sucrose, and auxin coordinately regulate berry ripening process of the Fujiminori grape. Funct. Integr. Genom. 1–17.
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. Genom. 10, 367–381.
Lai, B., Hu, B., Qin, Y.H., Zhao, J.T., Wang, H.C., Hu, G.B., 2015. Transcriptomic analysis of *Litchi chinensis* pericarp during maturation with a focus on chlorophyll degradation and flavonoid biosynthesis. BMC Genom. 16, 225–242.
Lecourieux, F., Kappel, C., Lecourieux, D., Serrano, A., Torres, E., Arce-Johnson, P., Delrot, S., 2013. An update on sugar transport and signalling in grapevine. J. Exp. Bot. 65 (3), 821–832.
Liang, Z., Wu, B., Fan, P., Yang, C., Duan, W., Zheng, X., Liu, C., Li, S., 2008. Anthocyanin composition and content in grape berry skin in *Vitis* germplasm. Food Chem. 111 (4), 837–844.
Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem. Soc. T 11 (5), 591–592.
Lurie, S., Ovadia, R., Nissim-Levi, A., Oren-Hamir, M., Kaplunov, T., Zutahy, Y., Weksler, H., Lichter, A., 2009. Abscisic acid improves colour development in 'Crimson Seedless' grapes in the vineyard and on detached berries. J. Hortic. Sci. Biotech. 84 (6), 639–644.
McGuire, R.G., 1992. Reporting of objective color measurements. HortScience 27 (12), 1254–1255.
Mohsen, A.T., 2011. Attempts to improve the berry quality and storability of grape "Crimson Seedless" with potassium compounds under desert conditions. J. Hort. Sci. Ornamen. Plants 3, 75–85.
Pfaffl, M., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, 2002–2007.
Peña-Neira, A., Cáceres, A., Pastenes, C., 2007. Low molecular weight phenolic and anthocyanin composition of grape skins from cv. Syrah (*Vitis vinifera* L.) in the Maipo Valley (Chile): effect of clusters thinning and vineyard yield. Food Sci. Technol. Int. 13 (2), 153–158.
Peppi, M.C., Fidelibus, M.W., Dokoozlian, N.K., 2006. Abscisic acid application timing and concentration affect firmness, pigmentation, and color of 'Flame Seedless' grapes. HortScience 41 (6), 1440–1445.
Peppi, M.C., Fidelibus, M.W., Dokoozlian, N.K., 2007. Application timing and concentration of abscisic acid affect the quality of 'Redglobe' grapes. J. Hortic. Sci. Biotech. 82, 304–310.
Peppi, M.C., Walker, M.A., Fidelibus, M.W., 2008. Application of abscisic acid rapidly upregulated UFGT gene expression and improved color of grape berries. Vitis 47 (1), 11–14.
Ramming, D.W., Tarailo, R., Badr, S.A., 1995. Crimson Seedless': a new late-maturing, red seedless grape. HortScience 30 (7), 1473–1474.
Singh Brar, H., Singh, Z., Swinny, E., 2008. Dynamics of anthocyanin and flavonol profiles in the Crimson Seedless grape berry skin during development and ripening. Sci. Hortic. 117, 349–356.
Spayd, S.E., Tarara, J.M., Mee, D.L., Ferguson, J.C., 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. Am. J. Enol. Vitic. 53, 171–182.
Szyjewicz, E., Rosner, N., Kliewer, W.M., 1984. Ethephon (2-Chloroethyl) phosphonic acid, Ethrel, (CEPA) in viticulture, a review. Amer. J. Enol. Viticult. 35, 117–123.
Villegas, D., Handford, M., Alcalde, J.A., Pérez-Donoso, A., 2016. Exogenous application of pectin-derived oligosaccharides to grape berries modifies anthocyanin accumulation, composition and gene expression. Plant Physiol. Biochem. 104, 125–133.
Wang, Z., Meng, D., Wang, A., Li, T., Jiang, S., Cong, P., Li, T., 2013. The methylation of the PcMYB10 promoter is associated with green-skinned sport in Max Red Bartlett pear. Plant Physiol. 162 (2), 885–896.
Wang, H., Huang, H., Huang, X., 2007. Differential effects of abscisic acid and ethylene on the fruit maturation of *Litchi chinensis* Sonn. Plant Growth Regul. 52 (3), 189–198.
Wei, Y.Z., Hu, F.C., Hu, G.B., Li, X.J., Huang, X.M., Wang, H.C., 2011. Differential expression of anthocyanin biosynthetic genes in relation to anthocyanin accumulation in the pericarp of *Litchi chinensis* Sonn. PLoS One 6 (4), e19455.
Xie, R., Zheng, L., He, S., Zheng, Y., Yi, S., Deng, L., 2011. Anthocyanin biosynthesis in fruit tree crops: genes and their regulation. Afr. J. Biotechnol. 10 (86), 19890–19897.
Yamane, T., Jeong, S.T., Goto-Yamamoto, N., Koshita, Y., Kobayashi, S., 2006. Effects of temperature on anthocyanin biosynthesis in grape berry skins. Am. J. Enol. Vitic. 57 (1), 54–58.
Zabala, G., Vodkin, L.O., 2014. Methylation affects transposition and splicing of a large CACTA transposon from a MYB transcription factor regulating anthocyanin synthase genes in soybean seed coats. PLoS One 9 (11), e111959.
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 Phys. 166 (12), 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 (6), 1579–1588.
Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L., Gruissem, W., 2004. GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol. 136 (4), 2621–2632.