Vitis **47** (1), 11–14 (2008)

Application of abscisic acid rapidly upregulated UFGT gene expression and improved color of grape berries

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Summary

The effect of abscisic acid (ABA) on the color of 'Crimson Seedless' grapes was linked to expression of the key anthocyanin pathway gene UDP-glucose:flavonoid 3-*O*-glucosyltransferase (UFGT). In the skins of ABA treated fruits, the level of mRNA UFGT increased markedly within one week and then returned to levels that were similar to those of non-treated fruits after three weeks. The mRNA UFGT levels from untreated fruits were similar from veraison to nine weeks later. The color of ABA treated fruit also changed quickly. One week after application, the skins of trated fruits had lower lightness (L*) and hue (h°), and they remained darker and more red-colored throughout the experiment.

K e y w o r d s : Abscisic acid, 'Crimson Seedless', UDP-glucose:flavanoid 3-*O*-glucosyltransferase, *Vitis vinifera* L.

Introduction

The color of red and black grapes is determined mainly by the anthocyanin and flavonoid content of their skins (Mazza 1995). Grape anthocyanin biosynthesis is regulated by Myb-like regulatory genes and by the UDP-glucose: flavonoid 3-*O*-glucosyltransferase (UFGT) gene (Boss *et al.* 1996 a and b, Kobayashi *et al.* 2002). The UFGT gene is present in all grapes, but is only expressed in red-fruit cultivars (Boss *et al.* 1996 b) and the temporal and spatial pattern of UFGT expression is limited to veraison and post-veraison tissues (Boss *et al.* 1996 a). Because the flavonoid pathway has many branches, increased expression of the regulatory genes may not cause anthocyanins to accumulate if other factors alter other branches (Boss *et al.* 1996 b). Moreover, the level of UFGT mRNA does not necessarily reflect UFGT enzyme levels or activities, but it may be suggestive of both, particularly when bolstered by other data such as pigment accumulation or color development.

Recently, Jeong *et al.* (2004) showed that application of abscisic acid (ABA) to 'Cabernet Sauvignon' (*Vitis vinifera* L.) berries stimulated the accumulation of mRNA coding for several enzymes involved in anthocyanin biosynthesis, including UFGT, between two and four weeks after application. Others have also confirmed that application of ABA may increase the anthocyanin content in the berry skins of other grape varieties (Lee *et al.* 1997, Peppi *et al.* 2006, 2007). The application times and concentrations of ABA that are most effective at enhancing color are not the same for each grapevine cultivar (Peppi *et al.* 2006, 2007), and the physiological basis for such differences is not known.

Application of 150 to 300 mg·l^{-1} ABA to 'Crimson Seedless' grapes at veraison can noticeably improve color within one week (Peppi, unpubl. data), which suggests that UFGT may be upregulated within days, rather than weeks, after application of ABA. The purpose of this experiment was to determine how different concentrations of ABA, applied to 'Crimson Seedless' grapes at veraison, affected the temporal expression of the UFGT gene and color development of the berries.

Material and Methods

Own-rooted mature and uniform 'Crimson Seedless' grapevines grown at the Kearney Agricultural Center, Parlier, California were used for the experiment. Vines were trellised on an open gable, trained to bilateral cordons and spur-pruned. Nine clusters of uniform size and at the same physiological stage were selected on each of five vines (replicates) and assigned to treatments. At veraison (July 12, 2005, 5-10 % berries softening) three clusters on each vine were subjected to one of three treatments: non-treated, sprayed to run-off with a solution of 150 mg·l^{-1}ABA, or sprayed to run-off with a solution of 300 mg·l^{-1}ABA. Samples of fruit were collected on five dates: before treatment application (day 0), one day, one week, three weeks and nine weeks after. On each sampling date two berries were randomly selected from each cluster (six berries per treatment per vine) and clipped using shears leaving the pedicel attached to the berries.

Immediately after sampling the color of the berries was measured with a colorimeter (CR-200, Minolta Inc., Ramsey, NJ, USA) in terms of lightness (L*), chroma (C*) and hue (hº) (McGuire 1992). Values for L* range from 0 (black, no light) to 100 (white); the C* scale starts at zero and higher values correspond to higher color intensity; and the angles for hº are arbitrarily assigned to red (0º), yellow (90º), green (180º) or blue (270º). Measurements of the color components were made on three equidistant points around the equator of each berry. Berries were then fro-

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zen at -20 ºC. Whole skins were peeled from the frozen berries, frozen in liquid nitrogen, weighed, and transported on dry ice to a laboratory at the University of California, Davis where they were stored at -80 ºC until use. Pulp from peeled berries was squeezed in cheesecloth and filtered, and homogenized juice was used for soluble solids and titratable acidity analyses. A temperature-compensating digital refractometer (Palette 101, Atago, Farmingdale, NY, USA) was used to measure soluble solids. Titratable acidity was calculated by the titration of juice diluted in deionized water (1:4 v/v) with 0.133 *N* NaOH to a pH 8.2 end point. Frozen skins were processed and analyzed with primers developed at the Lucy Whittier Molecular & Diagnostic Core Facility at the University of California, Davis. Grape skins were collected in 500 µl of nucleic acid purification lysis buffer (Applied Biosystems, Foster City, CA, USA) and stored at -20 ºC. Proteinase K and two grinding beads were added and the tissues homogenized in a GenoGrinder2000 (Spec-Certiprep, Metuchen, NJ, USA) for 2 min at 1000 strokes per minute. Protein digestion was at 56 ºC for 30 min and then the solution was put at -20 ºC for 30 min for RNA precipitation. The extraction of total RNA was done with an ABI 6700 automated nucleic acid (ANA) workstation (Applied Biosystems) as per manufacturer's instructions.

Total RNA was digested with DNase and then used to synthesize cDNA. The reverse transcription was done in 40 µl final volume using 100 units of SuperScript III (Invitrogen, Carlsbad, CA, USA), 600 ng hexadeoxyribonucleotide primers $(pd(N)_6)$, 10 U RNaseOut, and 1 mM dNTPs (Invitrogen). The reaction was maintained at 50 ºC for 120 min and was terminated with 60 µl water, 5 min at 95 ºC and cooling on ice. Quantification of mRNA was done by PCR reaction using TaqMan systems. Two primers and an internal fluorescent labeled Taqman probe (5´reporter dye FAM, 3´quencher dye TAMRA) were designed for each target gene (UFGT and 18 S ssrRNA) using a Primer Express software (Applied Biosystems). The forward UFGT 5´→ 3´primer sequence was GCCGGTT-GTACCCAACACAA and the reverse one was TGACG-GTGCCAAAGCTAATG. The 5´→ 3´ UFGT probe was CTGCCTCC. For 18S ssrRNA the forward primer was GGGTTCGATTCCGGAGAGG, the reverse primer was CCGTGTCAGGATTGGGTAATTT and the 5´→ 3´ probe was CCTGAGAAACGGCTACCACATCCAAGGA. Validation of the TaqMan PCR systems was done according to LEUTENEGGER *et al.* (1999) using a 2-fold dilution and analyzed in triplicate.

The PCR reactions were done with 20x primer (final concentration of 400 nM for each) and TaqMan probes (final concentration 80 nM) in 12 µl of a commercial PCR mastermix (TaqMan Universal PCR Mastermix, Applied Biosystems) containing 5 µl diluted cDNA per reaction. The mastermix also had 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 5 mM $MgCl_2$, 2.5 mM dNTP, 0.625 U AmpliTaq Gold DNA polymerase and 0.25 U AmpErase UNG. Amplification was done in an automated fluorometer (ABI PRISM 7900 HTA FAST, Applied Biosystems): 2 min at 50 ºC, 10 min at 95 ºC, 40 cycles of 15 sec at 95 ºC and annealing for 60 sec at 60 ºC. Fluorescent signals were collected during the annealing temperature.

Quantification of mRNA was done by the comparative cycle threshold (Ct) method (User Bulletin #2, Applied Biosystems) and reported as relative transcription. After normalizing Ct values with 18S ssrRNA the ΔCt was calibrated against the weakest signal of the target gene, calculated by $2^{-\Delta\Delta Ct}$ and then expressed as n-fold difference relative to the calibrator cDNA. Treatment means and standards of deviation were calculated on each harvest date and used for treatment comparisons.

Results and Discussion

One day after treatment, ABA-treated fruit had slightly higher levels of UFGT mRNA, but one week after treatment these differences were accentuated, and ABA concentration effects were clearly evident (Fig. 1). At that time, fruits treated with 150 mg·l⁻¹ABA had UFGT mRNA levels three times higher than the control, and fruits treated with 300 mg·l⁻¹ had UFGT levels six times higher. Three weeks after treatment, UFGT mRNA levels in treated fruits declined and became similar to, or slightly greater than, those of non-treated fruits. Levels of UFGT mRNA in control fruits were very similar from veraison to nine weeks later. The transient, but striking, increase in UFGT mRNA levels induced by ABA on Crimson Seedless differs somewhat from the effect on 'Cabernet Sauvignon' (JEONG *et al.* 2004), where UFGT mRNA increased and decreased over a period of weeks. Both findings support the notion that the natural increase in endogenous ABA levels at veraison may induce expression of the UFGT gene, which begins to be expressed at veraison (BOSS *et al.* 1996 a). However, the rapid response observed in Crimson Seedless is particularly important and makes this cultivar an interesting subject for future anthocyanin gene expression studies. Further work is needed to determine whether mRNA UFGT levels are controlled by changes in the expression of Myb genes or if some other factors may play a role.

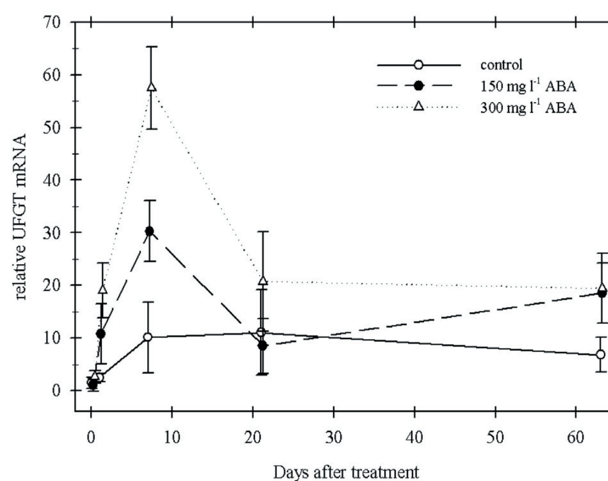

Fig. 1: Effect of abscisic acid (ABA) on skin mRNA levels of UFGT of 'Crimson Seedless' berries, 2005.

Color was strongly affected by ABA treatment and changes in color components were observed within one day of treatment (Fig. 2). Treatment effects on L* and hº were

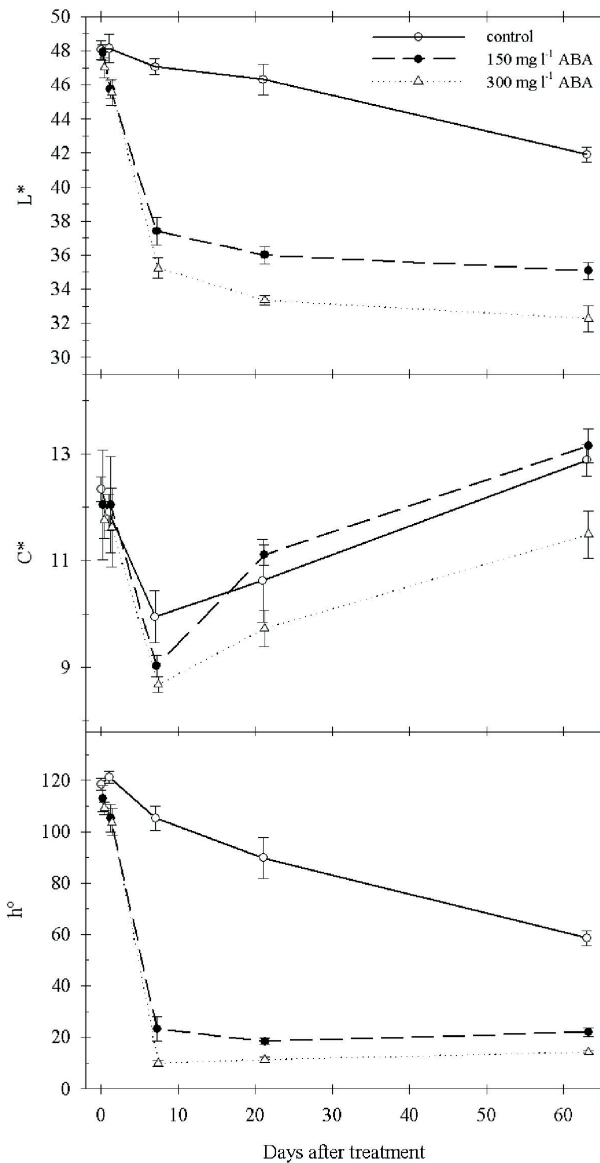

Fig. 2: Effect of abscisic acid (ABA) on the three components of color (lightness, L*; chroma, C*; and hue angle, h°) of 'Crimson Seedless' berries, 2005.

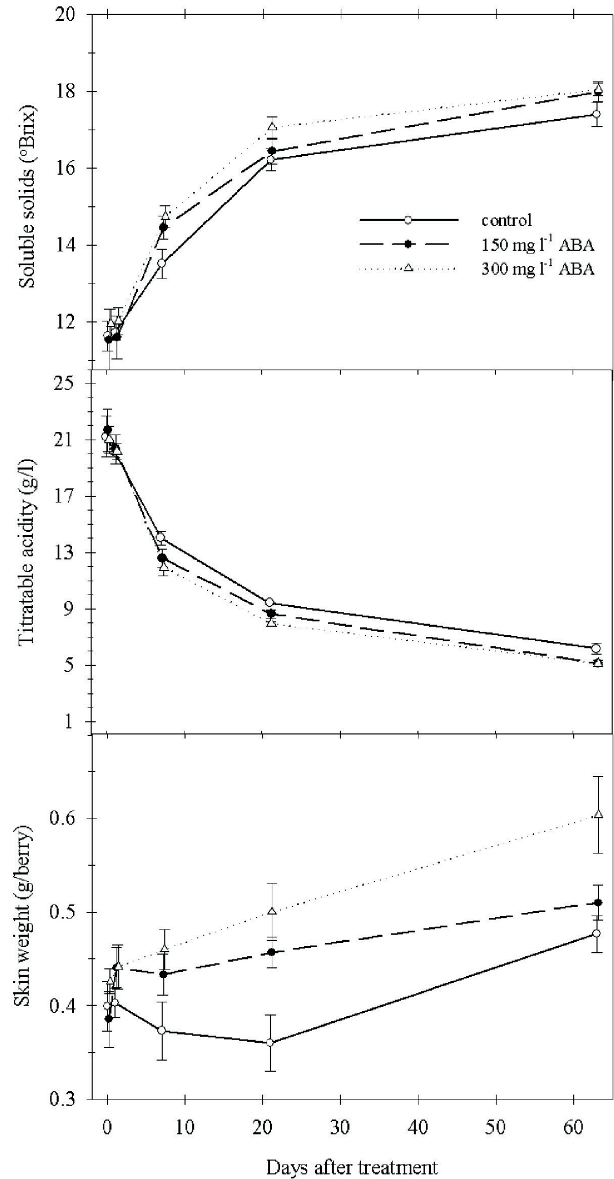

Fig. 3: Effect of abscisic acid (ABA) on soluble solids, titratable acidity and skin weight of 'Crimson Seedless' grapes, 2005.

very pronounced, but effects on C* were smaller and less consistent. One day after application ABA-treated fruits were darker and more red-colored than non-treated fruits and these differences were particularly evident when measured between one and three weeks post-veraison. The L* and h° of ABA-treated fruit did not change much between three and nine weeks after treatment whereas both variables continued to gradually decrease in control fruits. At the last sampling date control fruits remained less dark and red-colored than ABA-treated fruit. Thus, color changes in ABA-treated fruit were rapid and occurred shortly after application, while color changes in untreated control fruits were gradual. Changes in L* and h° were in general closely related to changes in mRNA levels coding for UFGT.

Application of ABA had little, if any, effect on fruit composition. Application of ABA slightly advanced maturity but changes were not enough to change probable harvest date of the fruit (Fig. 3). At one week and nine weeks after ABA application, ABA-treated berries had slightly higher soluble solids and lower titratable acidity than non-treated berries while three weeks after veraison non-treated fruit had slightly higher titratable acidity. By the last sampling date, all fruit had achieved the minimum sugar and sugar:acid ratio required, and application of ABA had little if any effect on fruit composition, as observed by others (Jeong *et al.* 2004, Lee *et al.* 1997, Peppi *et al.* 2006). There were no differences in the composition of fruits treated with different concentrations of ABA. However, skin fresh weight per berry, which generally increased throughout the experiment, was further increased by ABA treatment, especially in fruits treated with 300 $mg \cdot l^{-1}$ABA (Fig. 3). One week after treatment, skin weights of the ABA-treated fruits began increasing compared to the untreated control fruit, although nine weeks after treatment only fruits treated with 300 $mg \cdot l^{-1}$ABA still had greater skin weights than the control (Fig. 3). Even with the absence of differences in maturity, treatments showed differences in UFGT mRNA levels and in color. Thus, the effects of ABA treatment are

mostly limited to the peel. The increased peel fresh weight in ABA treated fruit could be the result of advanced skin maturation, consistent with ABA effects on skin pigmentation.

Acknowledgements

The authors thank K. Cathline and J. Osorio for technical assistance and funding from Valent BioSciences and the California Table Grape Commission.

References

Boss, P. K.; Davies, C.; Robinson, S.; 1996 a: 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**, 1059-1066.

Boss, P. K.; Davies, C.; Robinson, S.; 1996 b: Expression of anthocyanin biosynthesis pathway genes in red and white grapes. Plant Mol. Biol. **32**, 565-569.

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 berry skins. Plant Sci. **167**, 247-252.

Kobayashi, S.; Ishimuru, M.; Hiraoka, K.; Honda, C.; 2002: *Myb*-related genes of the Kyoho grape (*Vitis labruscana*) regulate anthocyanin biosynthesis. Planta **215**, 924-933.

Lee, K. S.; Lee, J. C.; Hwang, Y. S.; Hur, I. B.; 1997: Effects of natural type (S)-(+)-abscisic acid on anthocyanin accumulation and maturity in Kyoho grapes. J. Kor. Soc. Hort. Sci. **38**, 717-721.

Leutenegger, C. M.; Mislin, C. N.; Sigrist, B.; Ehrengruber, M. U.; Hofmann-Lehmann, R.; Lutz, H.; 1999: Quantitative real-time PCR for the measurement of feline cytokine mRNA. Veterin. Immunol. Immunopathol. **71**, 291-305.

McGuire, R. G.; 1992: Reporting of objective color measurements. HortScience **27**, 1254-1255.

Mazza, G.; 1995: Anthocyanins in grapes and grape products. Crit. Rev. Food Sci. Nutr. **35**, 341-371.

Peppi, M. C.; Fidelibus, M. W.; Dokoozlian, N.; 2006: Abscisic acid application timing and concentration affect firmness, pigmentation and color of 'Flame Seedless' grapes. HortScience **41**, 1449-1445.

Peppi, M. C.; Fidelibus, M. W.; Dokoozlian, N. K.; 2007: Application timing and concentration of abscisic acid affect the quality of 'Redglobe' grapes. J. Hort. Sci, Biotech. **82**, 304-310.

Received April 17, 2007