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Effects of abscisic acid treatment and night temperatures on anthocyanin composition in Pinot noir grapes

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Summary

Potted Pinot noir grapevines were grown under continuous high temperature (30 °C) or low night (15 °C) and high day (30 °C) temperatures after veraison. Half of the total number of clusters of each vine was sprayed with 250 ppm abscisic acid (ABA) at veraison. Anthocyanin accumulation in berry skins grown under high night temperatures was lower than that in berries grown under low night temperatures. HPLC analysis showed that the ratios of delphinidin-3-glucoside, cyanidin-3-glucoside and petunidin-3-glucoside to the total anthocyanin content were greatly reduced under high night temperatures. ABA treatment enhanced anthocyanin accumulation under high night temperatures to almost the same level as under low night temperatures; the ratio of each anthocyanin to the total anthocyanin, however, was not affected by ABA treatment.

K e y w o r d s : abscisic acid (ABA), anthocyanin composition, night temperatures.

Introduction

The anthocyanin content in grape berry skins is an important factor determining wine quality. Its accumulation in grape berry skins is generally influenced by temperature during the maturation period (Coombe 1987; Mullins *et al*. 1992). In warm regions, grape berries tend to be poorly coloured and night temperatures have considerable influence. As previously reported, high night temperatures also reduced the colouration of the Japanese table grape Kyoho (*Vitis labruscana* × *V. vinifera*) (Tomana *et al*. 1979). In future, global warming may affect grape colouration by elevated night temperatures in grape production areas that used to be suitable for adequate grape colouration. It has been reported that the application of abscisic acid (ABA), a phytohormone related to the ripening of berries (Coombe and Hale 1973), improved grape berry colouration (Kataoka *et al*. 1982 and 1984, Jeong *et al.* 2004). Tomana *et al*. (1979) reported that high fruit temperatures resulted in low ABA and anthocyanin concentrations in the berry skins. Kataoka *et al*. (1984) showed that ABA treatment of cv. Kyoho clusters enhanced anthocyanin accumulation in berry skins under high temperatures. However, the effects of ABA on the anthocyanin composition remain unclear. Anthocyanin composition is influenced by various factors, such as nitrogen (Hilbert *et al*. 2003), soil nutrients (Yokotsuka *et al*. 1999), cluster thinning (Guidoni *et al*. 2002), cluster shading (Gao and Cahoon 1994) and irrigation (Esteban *et al*. 2001). The anthocyanin composition as well as its content are important for wine quality because individual anthocyanins have different characteristics with regard to color or stability. *V. vinifera* cultivars accumulate several kinds of anthocyanins, *e.g.* 3-monoglucoside, 3-acetylglucoside and 3-*p*-coumaroylglucoside derivatives of delphinidin, cyanidin, petunidin, peonidin, and malvidin (Mazza and Miniati 1993). Pinot noir grape skins accumulate only 5 anthocyanins, namely, 3-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin (Fong *et al*. 1971). This simple anthocyanin profile is suitable for examining the effects of ABA and temperature on anthocyanin composition. In this study, we examined the effects of night temperature and ABA treatment on the anthocyanin composition.

Material and Methods

P l a n t m a t e r i a l a n d t r e a t m e n t s : Four 10-year-old potted Pinot noir grapevines (*Vitis vinifera* L.) grafted on SO 4 were grown in a phytotron. The vines were trained in Guyot trellising system. Each vine carried 15-16 clusters. At veraison (July 27), three clusters from each vine were harvested, and the vines were then divided into two groups that were grown under continous high (30 °C) or low night temperatures (15 °C). For both treatments daytime temperature was 30 °C (8 a.m.- 6 p.m.). Photoperiod corresponded to natural day length. In addition, half of the clusters of each vine was sprayed with 250 ppm abscisic acid (± ABA, Sigma, St. Louis, MO). The ABA solution was prepared by dissolving ABA in a minimal volume of 0.1 N KOH and by adding Tween 80 (0.1% final concentration) followed by dilution with distilled water. The pH of the solution was adjusted to 6.5-7.5 with hydrochloric acid. A total of 40 berries were sampled randomly at 12-d-intervals after treatment from each treatment plot: (1) low night temperatures (LNT), (2) low night temperatures with ABA treatment (LNT + ABA), (3) high night temperatures (HNT), and (4) high night temperatures with ABA treatment (HNT + ABA). Berry skins were peeled

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and immediately frozen in liquid nitrogen and stored at -80 °C until use. Total soluble solids of the berry juice was measured using a digital refractometer (PR-101; Atago, Tokyo, Japan). Titratable acidity was measured by titration of 1 ml of juice with 0.1 N NaOH to pH 8.2.

Anthocyanin extraction and HPLC analysis: The anthocyanin content was determined as described by ALI and STROMMER (2003) with some modifications. Two hundred and fifty mg of berry skin were ground with a mortar and pestle in liquid nitrogen and soaked in 5 ml of extraction buffer (formic acid/water/methanol (2:28:70/v:v)) for 24 h at 4 °C. The extract was then centrifuged at 15,000 x g for 5 min. The supernatant was filtered through a 0.45 µm filter and analyzed by HPLC (Shimadzu Scientific Instruments, Kyoto, Japan) consisting of an SPD-10A$_{VP}$ UV-VIS detector, SCL-10A$_{VP}$ system controller, dual LC-10AD$_{VP}$ pumps, a CR-7A PLUS integrator and a Zorbax SB-C18 (5 µm, 3.6 mm x 250 mm) column (Agilent Technologies, Palo Alto, CA). Solvent A consisted of 10 % formic acid in water and solvent B was acetonitrile. The solvent system was initially composed of 95 % A and 5 % B with the following steps: 5 min, 8 % B; 15 min, 9 % B; 20 min, 10 % B; 30 min, 20 % B; 35 min, 25 % B; 45-50 min, 30 % B. The flow rate was 1.0 ml·min^{-1} and the injection volume was 20 µl. Anthocyanins were quantified by peak area integration recorded at 520 nm using malvidin-3-glucoside as a standard; the concentration is expressed as malvidin-3-glucoside equivalents. Analyses were carried out in biological duplicates.

Results and Discussion

Effects on total soluble solids and titratable acidity: High night temperatures influenced maturation of Pinot Noir grape. Fig. 1 shows the effects of night temperatures and abscisic acid treatment on total soluble solids (A) and titratable acidity (B). The total soluble solids in HNT berries were lower than that in LNT berries after treatment. This result is in agreement with COOMBE (1987), who reported that with 30 °C day temperatures, low night temperatures (10 °C) resulted in a higher sugar content than warm nights, which may be associated with a higher translocation rate of sugar into berries. Titratable acidity in HNT berries was lower than that in LNT berries (Fig.1 B). COOMBE (1987) reported, that the titratable acidity of juice was negatively correlated with temperature and for this reason, malate is used as the substrate of respiration during ripening and that the respiration rate increased at high temperatures. In contrast, ABA treatment did not affect the total soluble solids content and titratable acidity in LNT and HNT berries, as previously reported by KATAOKA *et al*. (1982).

Effects on total anthocyanin concentration: The total anthocyanin concentrations in the skin of LNT and HNT berries increased after veraison and peaked 24 d after treatment (DAT; Fig. 2). Thereafter, they were slightly reduced and held constant in HNT and LNT, respectively. The total anthocyanin concentration in HNT berries was lower than that in LNT berries (Fig. 2), although the differences were not as remarkable as previously reported for cv. Kyoho (TOMANA *et al*. 1979). In HNT berries, the anthocyanin concentration was increased after application of ABA, which also increased the anthocyanin concentration in the skin of LNT berries. Under both conditions, the ABA treatment enhanced anthocyanin concentrations until 36 DAT, although the anthocyanin concentration in berries without ABA treatment reached peak level at 24 DAT. KATAOKA *et al*. (1984) has demonstrated that ABA treatment enhanced the anthocyanin accumulation in Kyoho berries under high temperatures (30 °C/25 °C, day/night temperature). The present results indicate that ABA treatment also enhances the anthocyanin accumulation in berries of cv. Pinot noir grown under high night temperatures.

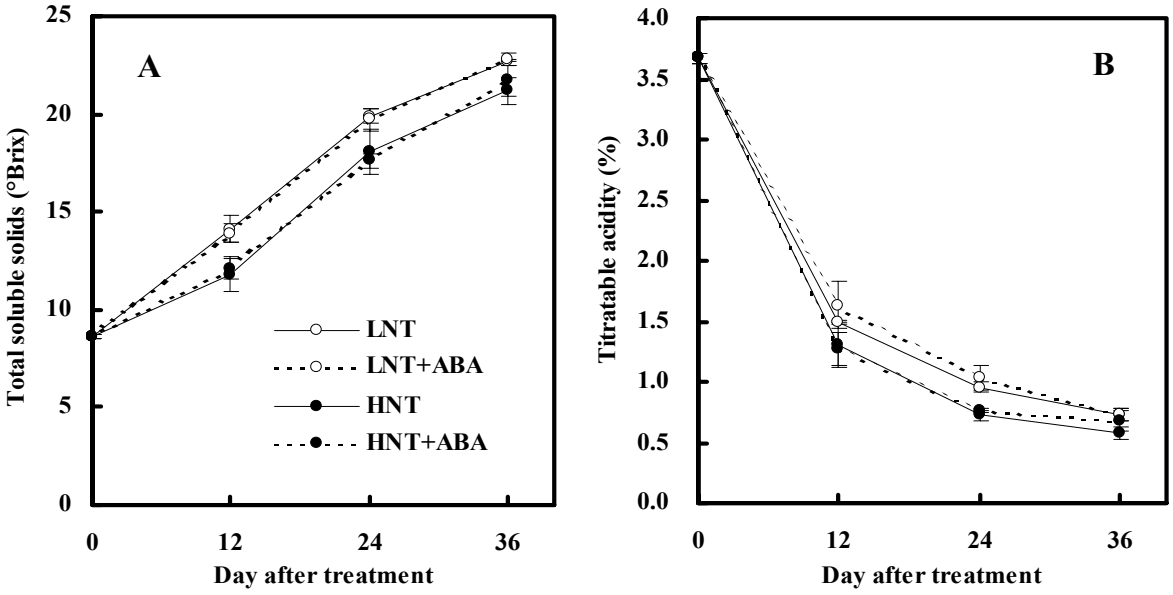

Fig. 1: Total soluble solids (**A**) and titratable acidity (**B**) in Pinot noir grape berries grown under low night temperatures (LNT), low night temperatures with ABA treatment (LNT +ABA), high night temperatures (HNT) and high night temperatures with ABA treatment (HNT + ABA). Vertical bars indicate standard deviation (n = 2).

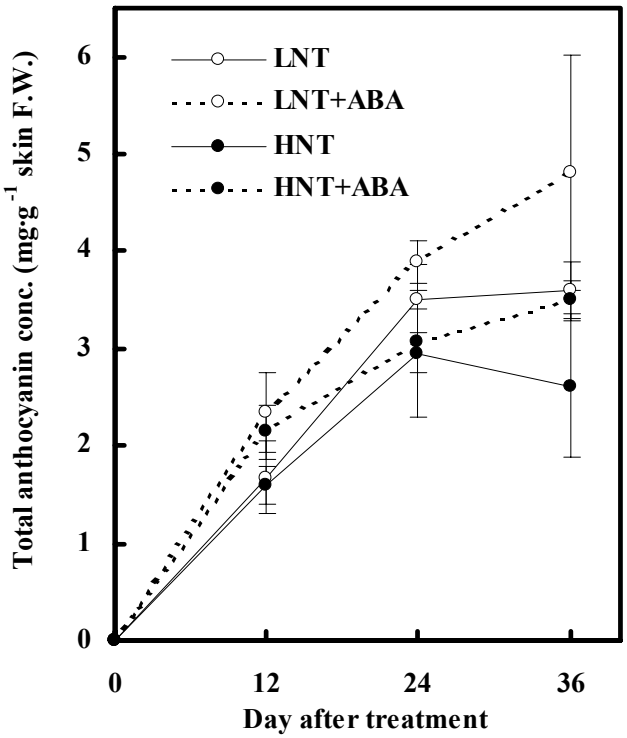

Fig. 2: Changes in the total anthocyanin concentration in the skin of Pinot noir grapes. For details see Fig. 1.

Effects on the anthocyanin composition: The effects of night temperature on the concentration of anthocyanins differed among individual anthocyanins. The concentrations of peonidin-3-glucoside and malvidin-3-glucoside, which are the main anthocyanins of Pinot noir, were not significantly influenced by either set of conditions (Fig. 3). On the other hand, concentrations of delphinidin-3-glucoside, cyanidin-3-glucoside, and petunidin-3-glucoside decreased markedly in HNT berries compared to LNT berries (Fig. 3). ABA treatment increased the concentrations of 5 individual anthocyanins, but HNT + ABA berries did not accumulate as much delphinidin-3-glucoside, cyanidin-3-glucoside, and petunidin-3-glucoside as LNT berries. Although no reports have been published on the effects of temperature on individual anthocyanin concentrations in grape skin, Dela *et al*. (2003) reported that the composition of anthocyanin underwent significant changes due to heat stress in rose. It has been reported that in grapes nitrogen availability affected the anthocyanin composition in Merlot (Hilbert *et al*. 2003). This report indicate that accumulation of malvidin-3-glucoside was the least sensitive to environmental conditions. The results agree in part with this study, which shows that decrease of rates of malvidin-3-glucoside and peonidin-3-glucoside in the skin of HNT berries was smaller than that of delphinidin-3-glucoside, cyanidin-3-glucoside, and petunidin-3-glucoside.

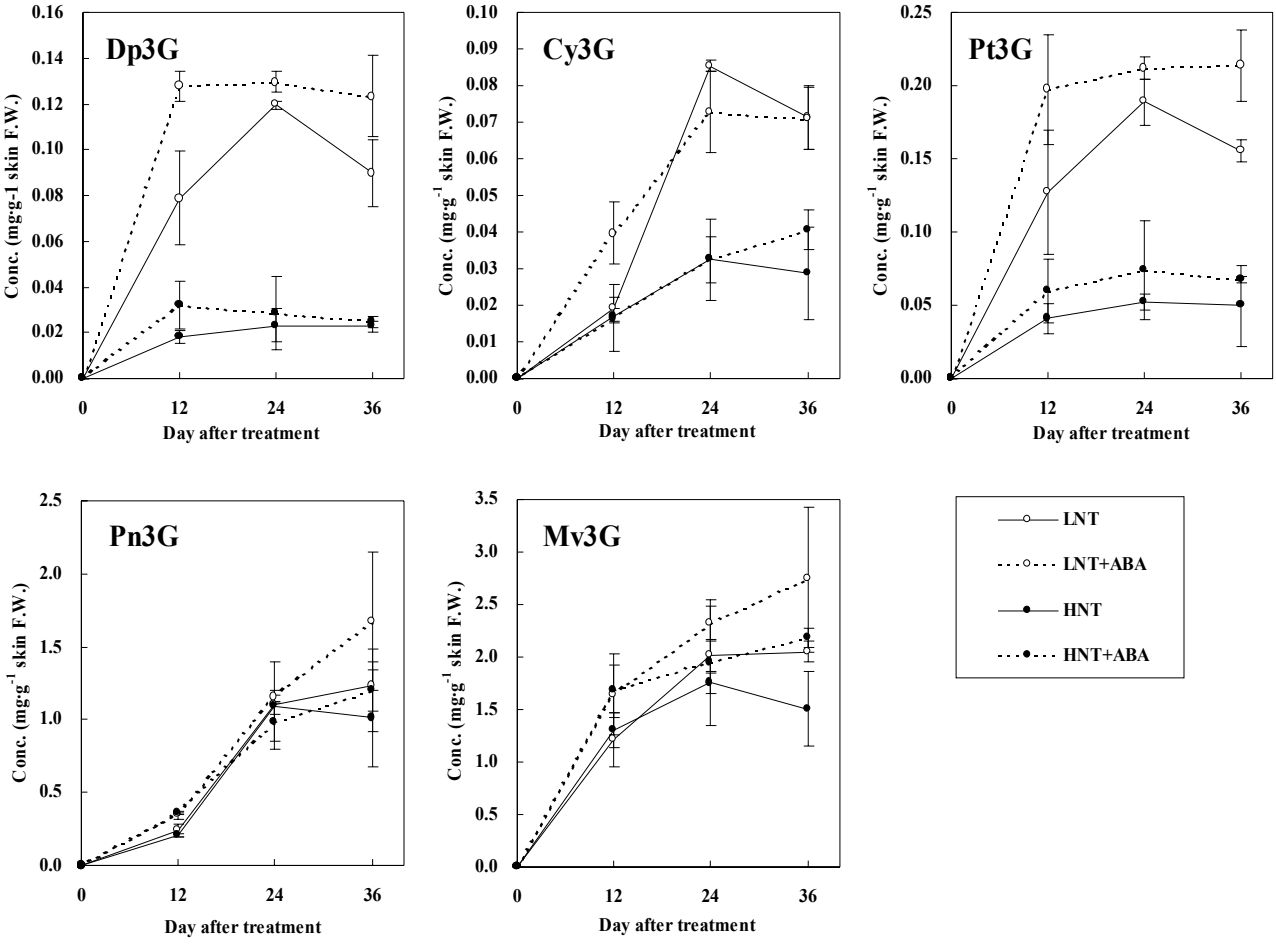

Fig. 3: Changes in the individual anthocyanin concentrations in the skin of Pinot noir grapes. For details see Fig. 1.

Malvidin and peonidin are highly methylated anthocyanidins of delphinidin and cyanidin derivatives (MAZZA and MINIATI 1993). In general, methoxylation, glycosylation and acylation lead to increased thermal stability of anthocyanin (JACKMAN and SMITH 1996). Thus, it is possible that low-methylated anthocyanins, delphinidin-3-glucoside, cyanidin-3-glucoside and petunidin-3-glucoside, degrade more easily under high temperatures than peonidin-3-glucoside and malvidin-3-glucoside. Another hypothesis that accounts for the low accumulation of delphinidin-3-glucoside, cyanidin-3-glucoside and petunidin-3-glucoside under HNT condition is that the anthocyanin methylation is enhanced. *E.g.* NAKAMURA *et al*. (1998) reported that anthocyanin methylation was enhanced by lower 2,4-dichlorophenoxyacetic acid (2,4-D) application in strawberry suspension culture and suggested that lower 2,4-D concentrations enhanced the activity of anthocyanin methyltransferase. Further research with regard to anthocyanin methyltransferase may clarify anthocyanin methylation at high temperatures.

The Table lists the ratio of individual anthocyanins to total anthocyanin in the skin of berries grown under LNT, LNT + ABA, HNT, and HNT + ABA conditions at 12, 24, and 36 DAT. The major anthocyanin is malvidin-3-monoglucoside, followed by peonidin-3-glucoside. The total of the other anthocyanins, delphinidin-3glucoside, cyanidin-3-glucoside and petunidin-3-glucoside, was approximately 10 % of the total anthocyanin. LNT and HNT berries differed in their ratio of individual anthocyanins to total anthocyanin. The ratios of delphinidin-3-glucoside, cyanidin-3-glucoside and petunidin-3-glucoside in HNT berries were significantly lower than in LNT berries at 12, 24 and 36 DAT (except for cyanidin-3-glucoside at 12 DAT). However, ABA treatment did not result in a significant difference in the ratio of individual anthocyanins to total anthocyanin because individual anthocyanins were increased at a similar rate by ABA treatment (Fig. 3). This is consistent with results of BAN *et al*. (1998), who stated that the anthocyanin composition in berry skins of Kyoho grapes is affected by shading treatments but not by the application of ABA. A mechanism for the increase of anthocyanin accumulation in grape skin by ABA treatment seems to involve the induction of *VvmybA1*, a putative regulatory gene of anthocyanin biosynthesis (KOBAYASHI *et al*. 2002). JEONG *et al*. (2004) demonstrated that mRNA accumulation of *VvmybA1* was affected by ABA in the same manner as mRNA accumulation of anthocyanin biosynthetic enzyme genes. This suggests that products of *VvmybA1* probably regulate the whole range of anthocyanin biosynthetic enzyme genes and not a specific enzyme gene. This could be the reason that ABA treatment enhanced anthocyanin concentration but has no effect on the anthocyanin composition in Pinot noir berry skin under high night temperatures.

Table

Anthocyanin composition in the skin of Pinot noir berries grown under low night temperatures (LNT), low night temperatures with ABA treatment (LNT + ABA), high night temperatures (HNT) and high night temperatures with ABA treatment (HNT + ABA)

Treatment	Dp3G	Cy3G	Pt3G	Pn3G	Mv3G
12 DAT					
LNT	4.7	1.1	7.5	14.5	72.2
LNT+ABA	5.5	1.7	8.4	15.1	69.4
HNT	1.1	1.1	2.6	13.2	82.0
HNT +ABA	1.5	0.8	2.8	16.8	78.1
Significance					
Temperature	***	ns	***	ns	**
ABA	ns	ns	ns	ns	ns
Interaction	ns	ns	ns	ns	ns
24 DAT					
LNT	3.4	2.4	5.4	31.5	57.3
LNT+ABA	3.3	1.9	5.4	29.8	59.6
HNT	0.8	1.1	1.8	36.8	59.5
HNT +ABA	0.9	1.1	2.3	32.6	63.1
Significance					
Temperature	***	**	***	ns	ns
ABA	ns	ns	ns	ns	ns
Interaction	ns	ns	ns	ns	ns
36 DAT					
LNT	2.5	2.0	4.3	34.2	57.0
LNT+ABA	2.6	1.5	4.5	34.6	56.8
HNT	0.9	1.1	1.8	38.3	57.9
HNT +ABA	0.7	1.2	1.9	34.0	62.2
Significance					
Temperature	***	**	**	ns	ns
ABA	ns	ns	ns	ns	ns
Interaction	ns	ns	ns	ns	ns

Mean of the ratio of individual anthocyanin to the total anthocyanin (%). DAT - days after treatment. ns, **, ***: non-significant at the 0.05 % level, significant at the 0.01 % and 0.001 % level, respectively using two-way analysis of variance. Abbreviations: Dp3G: delphinidin-3-glucoside; Cy3G: cyanidin-3-glucoside; Pt3G: petunidin-3-glucoside; Pn3G: peonidin-3-glucoside; Mv3G: malvidin-3-glucoside.

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