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Research Note

Nighttime temperature treatment of fruit clusters of 'Aki Queen' grapes during maturation and its effect on the skin color and abscisic acid content

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Introduction: In grapes, skin color is an important parameter of grape quality. The color in black and red grape skins is produced by anthocyanin accumulation, and the quantity and composition of the anthocyanins determine the skin color of the grapes (Shiraishi and Watanabe 1994). 'Aki Queen' grapes, which have a red skin, are a popular cultivar released by Japan's National Institute of Fruit Tree Science. However, especially in warm areas in Japan, the coloration of the skin is unsatisfactory, and this causes the market price to slump.

It is well known that low temperature and abscisic acid (ABA) promote grape coloration (Tomana *et al*. 1979, Kataoka *et al*. 1982); however, the relationship among coloration, temperature, and ABA content throughout the ripening stage of 'Aki Queen' has not been reported.

Therefore, our objective was to clarify the relationship among coloration, temperature of the cluster, and ABA content of 'Aki Queen' grape skins throughout the maturation period and to determine the effects of cluster temperature on grape coloration and ABA content.

Materials and Methods: Plant materials: Three-year-old 'Aki Queen' grapes (*V. labrusca* × *V. vinifera*) planted in 60-l plastic pots and grown in an unheated vinyl greenhouse were used. Full bloom was on May 20, and sampling dates were determined by the maturation stage: July 1, before veraison; July 14, start of veraison and onset of coloration; July 24, 10 d after onset of coloration; and August 7, 23 d after the onset of coloration and the date by which the total soluble solids (brix) and tartaric acid had reached a sufficient level for harvest. In the control clusters, the dates of veraison and the onset of coloration were 12 and 14 July, respectively.

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Temperature treatment: The temperature treatment was conducted by controlling the night (1800 to 0600 h) temperature at three levels: (1) high-nighttime-temperature, 5 °C higher than normal; (2) low-nighttime-temperature, 5 °C lower than normal; and (3) control, normal. The higher temperature than control was maintained by placing a heater around the clusters, and the lower temperature than control was maintained by using a vinyl tube to flow chilled water around the clusters. The heater was made from manganine wire (0.1-mm diameter), sandwiched by an internally insulated aluminum foil tape, and the degree of heating was controlled by a data logger (CR10X, Campbell Scientific, Logan, UT). Cooling was also controlled by the data logger. The temperature treatment was continued from July 1 to August 7. All clusters, as well as the wires and vinyl tubes used for temperature control, were covered with paper bags. Grapes in all three treatments were harvested on August 8.

Anthocyanin and ABA content in the berry skin: Anthocyanins were extracted from 1 g F.W. of skins with 5 ml of 50 % (v/v) acetic acid for 24 h at 4 °C; the extracts were then filtered, and absorbance was measured at a wavelength of 520 nm. Total anthocyanin concentrations were expressed as milligrams of cyanidin-3-glucoside (Extrasynthese, France) equivalents per gram of fresh weight. The extraction and purification of ABA from the grape skins were conducted according to the procedures of Koshita *et al*. (1999). Briefly, about 1 g F.W. of the samples was homogenized and extracted in 80 % acetone containing 100 $mg \cdot L^{-1}$ butylhydroxytoluene and subjected to solvent partitioning. The extracts were further purified by high-performance liquid chromatography. D_6-ABA was used as an internal standard for the ABA analysis, and the results were quantified by gas chromatography-mass spectrometry.

Results and Discussion: In the low-nighttime-temperature treatment, the anthocyanin concentration was higher than that in the control or high-nighttime-temperature treatments on July 14 and 24, but there was no difference in anthocyanin content between the control and low-nighttime-temperature treatments on August 7 (Figure). The ABA content of the berries in the low-nighttime-temperature treatment was about two times higher than in the high-nighttime-temperature treatment on July 14 and 24. On August 7, the ABA content was only slightly higher in the low-nighttime-temperature and the control treatments than in the high-nighttime-temperature treatment (Figure).

It has been reported that low temperature during maturation accelerates the coloration of the grape skin (Spayed *et al*. 2002), and that exogenously applied ABA promotes the coloration of grapes (Kataoka *et al*. 1982). On the other hand, the sugar content of grape berries is thought to be associated with the anthocyanin concentration in 'Cabernet Sauvignon' grapes (Hunter *et al*. 1991). Recently, cross-talk between a sugar-induced gene and an ABA signaling gene has been reported (Çakir *et al*. 2003). Therefore, it is important to grow grape vines under the same conditions, especially in studies of the grape coloration and temperature, to avoid loading different amount of photoassimilates

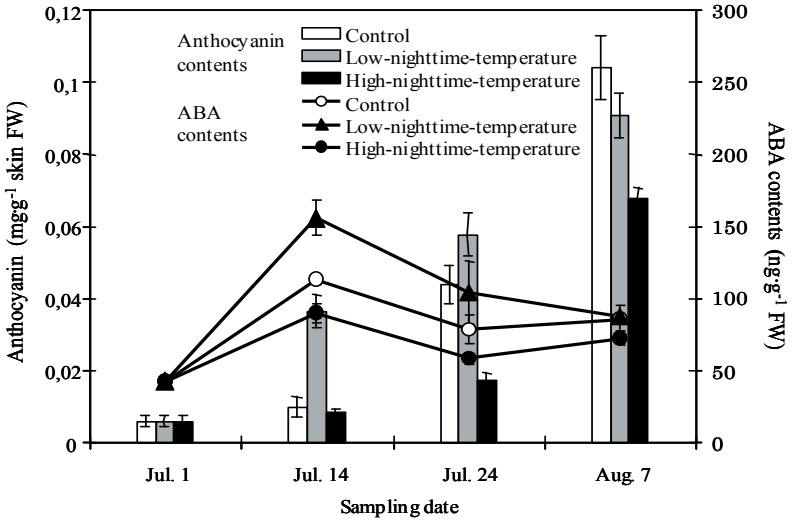

Figure: Anthocyanin and ABA content of grape skins in the normal, high-nighttime-temperature, and low-nighttime-temperature treatments. Vertical bars indicate SE (n = 3).

into grape berries. In our study, the leaf temperature of the grapevines was the same in all treatments, but the ABA content in the low-nighttime-temperature treatment was higher than those in the control and high-nighttime-temperature treatments. These results suggest that only differences in cluster temperature directly affected on the anthocyanin and ABA contents of the grape skin. TOMANA *et al*. (1979) also investigated the effect of different vine temperatures in combination with different grape cluster temperatures on 'Kyoho' grape ripening. They concluded that fruit temperature was the key factor that affected the ABA and anthocyanin contents of the grape berry skins by ABA and anthocyanin analysis at harvest time. We investigated the ABA content of the grape berry not only at harvest time but also during the grape maturation period, and the result showed that the ABA content just after veraison is the most important determinant of the anthocyanin content of 'Aki Queen' grape skins. Therefore, we conclude that the period between July 14 and July 24 is when ABA levels most strongly affect the anthocyanin content of grapes. This period extends from just after veraison until the time when half of the grapes developed coloration. YAMANE and SHIBAYAMA (2006) revealed that a period of 8 to 21 d after the onset of coloration was critical for the development of coloration in 'Aki Queen' grapes, and this time agreed well with our dates. Our results suggest that the ABA content of the grape skin might play an important role in skin coloration just after veraison of grape berry maturation.

Summary: We investigated the relationships among nighttime temperature and the coloration and abscisic acid (ABA) content of skins of 'Aki Queen' grapes (*Vitis labrusca* × *Vitis vinifera*) during the fruit maturation period to determine the effects of temperature on the development of skin color. High-nighttime temperature treatment from before veraison to harvest severely inhibited anthocyanin accumulation, when compared with the clusters of controls and low-nighttime temperatures. On the other hand, compare with the controls or with the high-nighttime temperature treatment, low-nighttime temperature treatment resulted in increased skin coloration or ABA content from veraison to 24 days after treatment. These data show that low temperatures at night accelerate anthocyanin biosynthesis and that the ABA content of the skin at the beginning of veraison is strongly associated with the development of grape coloration.

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