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Regulation of skin color in 'Aki Queen' grapes: Interactive effects of temperature, girdling, and leaf shading treatments on coloration and total soluble solids☆

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ABSTRACT

The interactive effects of temperature, girdling, and leaf shading treatments on the total soluble solids (TSS) content and coloration of 'Aki Queen' (*Vitis labruscana*) grapes were investigated. The TSS contents of berries were significantly higher in girdled plants than in plants with shaded leaves. Anthocyanin accumulation was greater at lower temperature (21 ± 2 °C) than at higher temperature (31 ± 2 °C) in both control and girdled plants, but anthocyanins did not accumulate in plants with shaded leaves at either temperature. Temperature did not influence TSS content. These results suggest that a high enough TSS content promotes anthocyanin synthesis under low temperature during maturation of 'Aki Queen' grape.

1. Introduction

The development of grape skin color is an important event both biologically and economically. Because the depth of color affects market price, growers must avoid poor coloration. Various environmental factors and culture treatments affect grape coloration. For example, low temperature during maturation enhances the development of the color and high temperature inhibits it (Tomana et al., 1979; Yamane et al., 2006; Koshita et al., 2007). Girdling of the trunk improves coloration (Fujishima et al., 2005; Yamane and Shibayama, 2006a, 2007), possibly by enhancing the total soluble solids (TSS) content of the berries. Fruit load also affects TSS and coloration of the berry (Takahashi, 1986). Shading of the leaves white-skinned grapes reduces TSSs (Kliewer et al., 1967; Kliewer and Antcliff, 1970). Defoliation also reduces TSS (Kliewer, 1970), because mature leaves produce the carbohydrates that comprise a large part of TSS.

'Aki Queen' a tetraploid with a red skin, good taste, and good texture, has difficulty developing the ideal skin color. To elucidate its coloring mechanism, researchers have investigated the relationship of skin color with fruit load (Kitamura et al., 2005) and number of leaves per fruit (Sato et al., 1997), the use of girdling treatment by examining treatment date and girdling width (Yamane and Shibayama, 2007), and the effect of high and low temperature on coloring stage (Yamane et al., 2006; Koshita et al., 2007). Although the individual effects of fruit load, girdling, and temperature have been characterized based on this research, their interaction of these factors has not been examined in 'Aki Queen'.

The abscisic acid (ABA) content of grape berries increases at the start of veraison (color development), and then gradually decreases (Coombe and Hale, 1973). The application of ABA to grape clusters enhances anthocyanin accumulation by activating the anthocyanin biosynthesis pathway (Ban et al., 2003). So ABA is believed to be a ripening hormone but there are no reports of ABA content under manipulated berry TSS content.

Here, we tried to elucidate the effects of TSS on grape coloration at high and low temperatures during the maturation period by manipulating TSS content of the berries by girdling and leaf-shading treatments.

2. Materials and methods

2.1. Plant materials

We used 2-year-old red grape (*Vitis labruscana* 'Aki Queen') vines grown in 20-L plastic pots in a sand compost mixture (9:1, v/v). A compound fertilizer containing 4.5 g N, 3 g P, and 4.5 g K,

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per vine was applied before bud break. Each vine was pruned to have only 1 fruit cluster (with approximately 30 berries), 12 main leaves, and 12 secondary leaves on the primary stem. We used three vines in each treatment. The vines were grown at the outdoors from before bud break until 14 July, and were then moved into growth chambers maintained at either $21 \pm 2\,^{\circ}C$ (low) or $31 \pm 2\,^{\circ}C$ (high). The developmental stages of the control grapes were as follows: 7 July, 35 days after full bloom and 2 weeks before coloring; 14 July, beginning of berry softening, but no coloration; 21 July, beginning of coloring; 28 July, half of the berries had developed color; 7 August, most berries had developed color; 13 August, harvest.

2.2. Manipulation of berry TSS contents

To increase the TSS content of the berries, we girdled the primary stem to a width of 1 cm from 10 cm to 30 cm above the soil to remove the phloem. To decrease TSS, we covered all of the leaves with paper bags wrapped in aluminum foil. The leaves were covered until harvest. Treatments were applied on 7 July 2006, 35 days after full bloom, which we previously found to be the most effective timing for enhancing anthocyanin accumulation by girdling (Yamane and Shibayama, 2007).

2.3. Temperature treatment

Temperature treatments began on 14 July, 1 week before coloring, when coloring is most sensitive to temperature (Yamane and Shibayama, 2006b). All vines were transferred into the growth chambers and were then grown until harvest on 13 August.

2.4. Analysis of TSS, anthocyanin contents, berry weights, and total acidity

Berries were picked on 14 July, 28 July, and 7 August to determine the TSS and anthocyanin content. Because the berries of 'Aki Queen' do not show synchronized color development even in the same cluster, we sampled three moderately colored berries per cluster. The TSS content was determined with a digital refractometer (PR-100, Atago Co., Tokyo, Japan). Anthocyanins were extracted from 1 g (fresh weight) of berry skins in 5 mL 50% (v/v) acetic acid for 24 h at $4\,^{\circ}C$. The absorbance of the extracts was measured at 520 nm. Total anthocyanin contents were expressed as micrograms of cyanidin-3-glucoside (Extrasynthèse, Genay, France) equivalent per square centimeter of skin. On 13 August, berries were harvested to determine their weight, TSS, and total acidity. The total acidity was determined by titration and expressed as the mass (g) of tartaric acid equivalent per 100 mL of juice.

2.5. Analysis of abscisic acid (ABA) content

The ABA contents of the skins were determined on 28 July and 7 August by the methods of Setha et al. (2004), with some modifications. The frozen skins were homogenized with a known amount of hexadeuterated (d6) ABA in 80% methanol and then filtered. The residue was re-extracted twice with 80% (v/v) methanol. The filtrate was reduced to an aqueous residue *in vacuo*, and the pH was adjusted to 2.5 by adding 0.1 N HCl. The aqueous solution was extracted three times with ethyl acetate, and the extract was evaporated to dryness. The residue was dissolved in 80% methanol, applied to a Sep-Pak cartridge (Waters Milford, MA, USA) and eluted with 80% (v/v) methanol. The eluate was evaporated to dryness, and the residue was redissolved in 25% (v/v) aqueous acetonitrile containing 25 mM acetic acid, and was then separated by high-performance liquid chromatography (ODS column, TSK-GEL ODS-100Z, Tosoh, Tokyo, Japan, 250 mm × 4.6 mm i.d., $40\,^{\circ}C$; flow rate of 1.3 mL min^{-1}; solvent A, 25% (v/v) acetonitrile with 20 mM acetic acid; solvent B, 80% (v/v) acetonitrile with 20 mM acetic acid; gradient profile 0–20 min, 0–100% B; 20–30 min, 100% B; 30–35 min, 100–0% B). The retention time of the ABA was 10 min. The ABA fraction was methylated with ethereal diazomethane and quantified by means of gas chromatography–mass spectrometry in selected ion monitoring mode.

Table 1
Total soluble solids (TSS) content of 'Aki Queen' grapes on 14 July, 7 days after the girdling and leaf shading treatments were applied.

Treatment	TSS (°Brix)
Control	9.2 b
Girdled	11.5 a
Leaf-shaded	6.9 c

Values are the means of three replicates. Values within a column followed by different letters differ significantly (Tukey's test, $P < 0.05$).

3. Results

3.1. Effects of girdling, leaf shading, and temperature treatments on berry TSS content

At 7 days after the girdling and shading treatments were applied, the TSS content of the berries of the girdled vines was significantly higher than that of the control plants, which was significantly higher than that of the plants with shaded leaves (Table 1). At 14 days after the temperature treatments began, the results were the same at both temperatures (Table 2). At 24 days, the TSS contents of the girdled and control plants did not differ significantly, but they were significantly higher than those of the shaded plants at both temperatures (Table 3).

3.2. Effects of girdling, leaf shading, and temperature treatments on berry anthocyanin content

At 14 and 24 days after the temperature treatments began, the anthocyanin content in the girdled low-temperature treatment was significantly higher than that in all other treatments which were not significantly different (Tables 2 and 3).

3.3. Effect of girdling, leaf shading and temperature treatments on berry ABA content

At 14 days after the temperature treatments began, the ABA content in shaded plants was significantly less than that in girdled plants at both temperatures, but temperature had no effect (Table 2). At 24 days, there was no significant difference in the ABA content in any of the treatments (Table 3).

3.4. Effect of girdling, leaf shading, and temperature treatments on fruit quality

At harvest, on 13 August, there was no significant difference in berry weights among the treatment combinations (Table 4). The TSS content showed the same trends as those shown in Tables 2 and 3 (i.e., the highest TSS content was found in the girdling treatment at both temperatures). Acidity was significantly higher in the shading treatment at both temperatures, but it was higher at low temperature. Shading therefore, delayed the decrease in acidity during berry maturations at both temperatures. Within each treatment, high temperature significantly decreased the acidity.