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Impact of temperature and sunlight on the skin coloration of the 'Kyoho' table grape

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ABSTRACT

We evaluated the impact of increased temperature and sunlight exposure, features of global warming, on the skin coloration of 'Kyoho' table grape berries. Skin coloration, anthocyanin accumulation, abscisic acid (ABA) content, and transcript levels of anthocyanin biosynthetic genes in the skins were examined during the ripening season under continuous 24 °C, 27 °C and 30 °C temperatures together with a sun or shade treatment. Skin coloration and anthocyanin accumulation were sufficient in the berries of plants ripened at 24 °C with a sun-exposure treatment. They also had higher ABA contents and anthocyanin biosynthetic gene transcript levels than grapes under higher temperatures. The skins of grapes ripened at 27 °C or 30 °C showed insufficient coloration and low levels of anthocyanin accumulation, ABA content, and anthocyanin biosynthetic gene transcripts levels, irrespective of the light conditions. These results suggest that temperatures exceeding 27 °C during the ripening season lead to insufficient berry coloration as a result of low levels of ABA and anthocyanin biosynthetic gene expression levels.

1. Introduction

Viticulture is a key economic activity in many regions of the world that is at risk because of climate change, known as "global warming" (Jones and Webb, 2010). In Europe, the growing – season mean temperatures increased by about ~1.3 °C from 1950 to 1999 and by ~1.7 °C from 1950 to 2004 (Jones et al., 2005). Climate changes related to weather patterns and carbon dioxide levels may cause shifts in grape chemistry. In particular, higher temperatures under a future warmer climate would inhibit the formation of anthocyanin in the berries, resulting in the worldwide reduction of wine grape coloration (Downey et al., 2006; Fraga et al., 2012; Mozell and Thach, 2014). In Japan, the impact of global warming on table grape production is significant in grapes grown in the southwestern area, resulting in poor skin coloration (Mori et al., 2005; MAFF, 2007). As shown in Fig. 1, the coloration of black-skinned 'Kyoho' grape (*Vitis labruscana* L. × *Vitis vinifera* L.), the leading table grape cultivar in Japan, during the ripening season (July to early August) has been slowly but steadily inhibited by the upward (+2.1 °C) shift of the average temperature over the past two decades.

Skin coloration is a major factor determining the fruit quality of grapes and is attributable to the accumulation of anthocyanin pigments (Boss et al., 1996). The anthocyanin accumulation in the berry skins is highly regulated in response to environmental factors, such as temperature, light, nutrient, plant hormone, and irrigation (Kliewer, 1970; Jeong et al., 2004; Boss and Davies, 2009). Of these factors, since the 1960s, the effects of temperature and light on the anthocyanin accumulation have been extensively investigated (reviewed by Downey et al., 2006). It appears that exposing whole vines or clusters to a low temperature and high light intensity enhances anthocyanin accumulation, while a high temperature and/or shading treatments reduces berry skin coloration. Particularly, reports of increasing anthocyanin accumulation due to sunlight exposure have helped growers to avoid poor skin coloration by incorporating light quality control methods, such as shoot positioning, light reflectors, and partial defoliation, around the fruiting zone. However, these approaches produced contradictory results, which may be explained by the different cultivars, locations, seasons, sampling times, and treatment periods. Furthermore, few reports have investigated the temperature threshold for insufficient skin coloration in table grapes in association with global warming.

In a series of phytotron studies on potted 'Kyoho' grapevines from 2009 to 2011, we have evaluated the impact of temperature and sunlight on the berries' skin coloration. Temperature-control experiments in 2009 showed that a low-temperature treatment

Abbreviations: ABA, abscisic acid; SSC, soluble sugar content; FW, fresh weight; UFGT, UDP Glc-flavonoid 3-*O*-glucosyltransferase.

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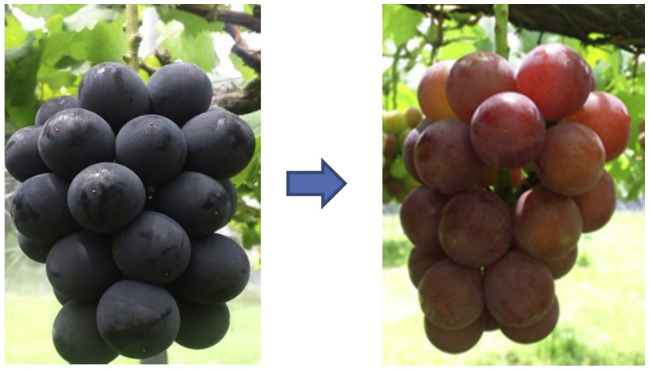

Fig. 1. Shift in skin coloration of 'Kyoho' berries during the ripening season from 1992 to 2012 at Fukuoka Agricultural Research Center, Fukuoka, Japan (33°50′-N, 130°57′-E).
Average temperature during ripening season (July to early August): 25.7 °C in 1992 and 27.8 °C in 2012.

(25 °C day/20 °C night) resulted in a darker skin coloration than those of high-temperature treatments (35 °C day/30 °C night and 30 °C day/25 °C night) (Table S1). In addition, a low-temperature treatment from 0 to 25 days after véraison resulted in a darker berry skin than a treatment from 26 to 50 days after véraison. In 2010, high temperature (35 °C day/25 °C night and 30 °C day/25 °C night) and shading treatments significantly inhibited the skin coloration (Table S2). The skin coloration in 2011 was suppressed by a high night temperature (30 °C) and shading treatments (Table S3). The skin coloration could be recovered by a low night temperature of 20 to 25 °C, as demonstrated by Mori et al. (2005). Additionally, field observations from 1993 to 2008 indicated that the skin coloration of 'Kyoho' grapes during the ripening season tended to be insufficient (below 8 on the color chart) at the intersection of an average temperature of ~27 °C and an average of ~200 h of sunshine (Fig. S1).

These observations suggested that the temperature threshold for insufficient skin coloration in table grapes exists between 25 °C and 30 °C. Thus, the objectives of this study were to (1) evaluate the temperature and light effects on skin coloration of 'Kyoho' berries during the ripening season, and (2) estimate the temperature threshold for insufficient skin coloration, with respect to consumer acceptance.

2. Materials and methods

2.1. Plant material and growth conditions

We used 3-year-old potted 'Kyoho' grape vines (*V. labruscna* L. × *V. vinifera* L.) in this study. In 2012, 24 vines were grown in a sideless plastic house at Fukuoka Agricultural Research Center, Fukuoka, Japan (33°50′-N, 130°57′-E). Each vine had three grape clusters, which were dipped in a solution containing 25 mg L^{-1} gibberellic acid (GA_3) and 5 mg L^{-1} forchlorfenuron 3 days after full bloom (25 May). On 28 June 2012, the potted vines were transferred into a phytotron in which the temperature and shading treatments were applied. The vines were subjected to one of six combinations of temperature and light conditions ($n = 4$ vines per treatment). The temperature conditions were as follows: high (continuous 30 °C), moderate (continuous 27 °C), and low (continuous 24 °C). The light conditions were as follows: shade (vines covered with Kuremona Cheese Cloth, which absorbs 60% of incidental solar radiation) and sun-exposed (vines left uncovered). Berry sampling commenced on 28 June, and the subsequent sampling was conducted at 14 days intervals (12 July, 26 July, and 8 August).

2.2. Anthocyanin concentration and berry composition

Four berries per vine were weighed, and their skin color was measured using a color chart for black grapes (National Institute of Fruit Tree Science, Japan), and then they were frozen at −80 °C until required. Skin color was scored from 0, indicating a green skin, to 12, indicating a black skin.

Anthocyanin was extracted from frozen berry skins in 10 mL 50% (v/v) acetic acid for 24 h at 4 °C according to Shiraishi et al. (2007). The absorbance of the extract was then measured at 520 nm (A_{520} g^{-1} skin) using a spectrophotometer (UV-260, Shimadzu, Kyoto, Japan). Ten berries from each vine were sampled on 8 August. Five berries were weighed, their skin color measured, and then the berries were crushed. The juice samples were immediately analyzed to determine the soluble sugar content (SSC) with a hand refractometer (N1, ATAGO, Tokyo, Japan). Titratable acid content of the juice was measured by the method of Shiraishi et al. (2010). The skin colors of the remaining five berries were measured, and then they were frozen at −80 °C until anthocyanin, abscisic acid (ABA), and gene transcript levels were measured.

2.3. ABA

ABA was extracted from 1 g frozen berry skins in 100 mL of methanol for 24 h at 4 °C. Then, 1 mL extract was mixed 4 mL 80% methanol, and the mixture was concentrated to 1 mL by centrifugation. The concentrated liquid was diluted to 50 mL with distilled water. The ABA content in the diluted solution was measured using an ABA Immunoassay Detection Kit (Sigma–Aldrich, St. Louis, MO, USA) as described in the manufacturer's manual.

2.4. Anthocyanin biosynthetic gene transcript levels

Total RNA was isolated from the frozen skins of berries collected on 12 July and 8 August. The RNA was isolated using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. cDNAs were synthesized using Prime Script RT Master Mix (Takara, Shiga, Japan) as described in the manufacturer's manual. We analyzed the transcript levels of a *MYB*-related gene (*VIMYBA2*; Azuma et al., 2011) and an anthocyanin biosynthesis-related gene (*UFGT: UDP Glc-flavonoid 3-O-glucosyltransferase*; Jeong et al., 2004) by quantitative real-time PCR (qRT-PCR) using a 7500 Real-Time PCR system (Applied Biosystems, FosterCity, CA, USA) and SYBR Premix Ex Taq II (Takara) as described in the manufacturer's manual. We conducted a qRT-PCR analysis for two replicates per prepared cDNA sample, and the transcript levels of each gene were normalized to that of *VvUbiquitin1* (Bogs et al., 2006).

3. Results

3.1. Skin coloration and anthocyanin accumulation

The skin coloring of the berries under different temperature and light conditions occurred gradually after 2 weeks of treatment (12 July), and steadily increased until 8 August (Fig. 2). Also, the softening of the most berries was visually observed on 12 July, corresponding to the onset of stage III (véraison) in grapevine growth (Mullins et al., 1992). At harvest (8 August), berries in the low-temperature (24 °C)/sun-exposed treatment showed the highest color (10.0), while the lowest color (0.1) occurred in the high-temperature (30 °C)/shaded treatment. At the same temperature, the color was always darker for sun-exposed berries than for shaded ones. Generally, color scores >8 (violet–black) and <7 (red–violet) indicate sufficient and insufficient coloration, respectively, for market use in Japan. The color score of sun-exposed