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Effects of temperature during fruit growth and maturation on pigmentation of purple cherry tomato containing a functional anthocyanin

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Abstract

Anthocyanins are natural pigments distributed widely among higher plants. Anthocyanins in plant-derived food are also important nutrient constituents, due to their dietary health benefits. Tomato (*Solanum lycopersicum* L.) is an important vegetable crop grown worldwide, many with anthocyanins in their fruit tissues, but the pigmentation varies specially in the popular purple tomato. To develop tomatoes with a high anthocyanin content, we conducted cultivation experiments under natural temperature and sunlight conditions using two purple cherry tomato cultivars 'Toscana Violet' and 'Blue Bingo' and an ordinary cherry tomato cultivar 'Amakko' as a control to clarify problems which we may encounter during year-round cultivation of purple tomatoes. The content of anthocyanins was high in the tomatoes cultivated in the winter but extremely low in those cultivated in the summer. These findings suggested that the high temperature in summer may cause poor anthocyanin production resulting in less pigmented fruits, although the yield of fruits itself is not be affected greatly. Furthermore, treatment of 'Blue Bingo' fruits at late growth and maturation stage by a night (8 h)-and-day (16 h) temperature regime of 15-22 °C resulted in marked accumulation of anthocyanins in skin and outer pericarp of the fruits, but those of 20-27 °C and 25-32 °C had no or little accumulation.

Key words: *Solanum lycopersicum*, purple tomato, anthocyanin accumulation, high temperature, year-round cultivation.

Introduction

Anthocyanins are natural pigments widely distributed in higher plants (Grotewold, 2006). Anthocyanins in plant-derived food are also important nutrient constituents, due to their dietary health benefits (Buer *et al.*, 2010; De Pascual-Teresa *et al.*, 2010; Eugenio *et al.*, 2008; Spencer, 2010). The biological effects of anthocyanins have been attributed to their antioxidant activity (Pietta, 2000).

Cultivated tomatoes (*Solanum lycopersicum* L.), one of the most important food crops in the world (Bovy *et al.* 2002; Willits *et al.* 2005), do not usually produce anthocyanins in their fruit tissues, although they do in vegetative tissues. Some wild tomato relatives bear anthocyanin-containing fruits, which are characterized by purple color in skin and outer pericarp of their fruits. This purple trait has been successfully transferred into the fruits of cultivated tomatoes after introgression of *Aft* (*Anthocyanin fruit*) gene by an interspecific cross with *S. chilense* Dunal (Giorgiev, 1972), and that of *Abg* (*Aubergine*) from *S. lycopersicoides* Dunal (Rick *et al.*, 1994). In *Aft* fruits of cultivated tomatoes, anthocyanins are produced in the subepidermal tissue from the green stage resulting in a purple skin color. Anthocyanidins produced in *Aft* fruit are composed mainly of petunidin, malvidin, and delphinidin (Jones *et al.*, 2003), which are similar in composition and quantity to those from vegetative tissues (Ibrahim *et al.*, 1968). *Aft* requires high light intensity to induce anthocyanin expression in fruit tissues (Mes *et al.*, 2008). On the other hand, the introgression of *atv* (*atroviolacium*) gene from *S. cheesmaniae* (L. Riley) Fosberg also caused anthocyanin expression in cultivated tomato fruits (Rick, 1964). Anthocyanin expression by *atv* is different from that by *Aft*; purple color appeared in the entire plants and more in leaves and stems than fruits (Gonzali *et al.*, 2009).

A 'purple' tomato, highly enriched with anthocyanins throughout the fruit tissues was recently produced by the ectopic expression of two transcription factors, Delila and Rosea1 from snapdragon (*Antirrhinum majus* L.) in 'MicroTom' tomato (Butelli *et al.*, 2008). However, the fruit is not tasty since the parent 'MicroTom' tomato is not palatable, and it has low productivity (less than 10 fruits per plant). Furthermore, since it is genetically modified, it would require much time and cost before approval as food. Therefore, there is an ongoing interest in breeding tomato cultivars with a high anthocyanin content without genetic engineering (Willits *et al.* 2005), and developing a cultivating method(s) to increase the anthocyanin content in fruits.

In the present study, we conducted cultivation experiments of non-transgenic purple cherry tomatoes for development of cultivation method to harvest the fruits year-round. We found that the purple pigmentation of the tomato fruit was greatly affected by the temperature during fruit maturation.

Materials and methods

Tomato seeds: Two cultivars of cherry tomato (*Solanum lycoperiscum* L.), 'Toscana Violet' and 'Blue Bingo', with purple fruits, and one cultivar 'Amakko' with ordinary fruits without purple pigmentation, as a control, were used in this experiment. Seeds of 'Toscana Violet' were purchased from Pioneer EcoScience Co., Ltd. Tokyo, those of 'Blue Bingo' from

Sole Seed Co., Tokyo, and those of 'Amakko' from Marutane Co., Ltd., Kyoto in the early spring of 2014.

Plant growth and fruit production

Experiment 1. The experiment was carried out at The University Farm of Kyoto Prefectural University in Seika Town, Kyoto Prefecture, Japan (N30°45' and E135°47'). In the experiment with organic-medium culture, tomato seeds were sown in a plastic germination tray filled with a germination mix (Takii & Co., Ltd, Kyoto, Japan) in a glass house. The seeds were germinated in the dark, and seedlings were grown in the glass house under natural diurnal temperature regime and natural day light for 3 to 4 weeks with necessary irrigation. Plants were transplanted to a culture mix (Takii & Co., Ltd; one plant per 20 L culture mix supplemented with N 300, P 470 and K 390 mg/L). They were grown in a greenhouse and maintained at a minimum temperature of 15 °C in the winter season. Changes in temperature in the greenhouse were monitored continuously during cultivation.

Plants were pinched off below the fourth truss, leaving three flower clusters, and all lateral shoots were removed. At anthesis, the flower trusses were sprayed with 4-chlorophenoxyacetic acid (Tomato Tones; Nissan Chemical Industries, Ltd, Tokyo, Japan). Seven to ten replicated plants were grown per cultivar. The plants were cultivated from April 2014 to March 2015, that is from April to July (first cultivation), from June to October (second cultivation) and from October to March (third cultivation).

Three mature fruits per truss were harvested in the order of maturation, resulting in nine fruits in total per plant. However, the total number of harvested fruits varied with the cultivar and cultivation experiment. Many plants did not survive the hot summer season and fruit drop occurred by an unknown cause.

Each fruit was weighed, and its diameter was determined by averaging diameters measured in two opposite directions on the hypothetical equatorial plate. Color of fruit skin was categorized by observation with the naked eye. The fruits were then stored at –80°C until assay of anthocyanin content.

Experiment 2: The experiment was carried out at Faculty of Agriculture, Ryukoku University in Otsu city, Shiga Prefecture, Japan (N34°57' and E135°56'). 'Blue Bingo' seeds were sown on May 8, 2015 and grown thereafter as described above. Plants were transplanted into the culture mix in plastic pots (30 cm in diameter, 14 L culture mix, one plant per pot), and grown further in a glass house, and plants were pinched off below the third truss, leaving two flower (fruit) clusters. On July 8, 2015, when the initial three fruits at the first fruit cluster became around 2 cm in diameter at the late stage of fruit growth and maturation, plants were transferred to growth chambers (LPH-350S, Nippon Medical & Chemical Instruments, Osaka, Japan) set at night (8 h)-and-day (16 h, 100 μmol m^{-2} s^{-1} by white fluorescent lamps) temperature regimes of 15-22, 20-27 or 25-32 °C. The plants were grown for one week, and fruits were harvested, inspected and photographed. Then, the harvested fruits were left at room temperature for two weeks, during which period their photographs were taken every week.

Anthocyanin determination: Frozen fruits were pulverized with a chilled pestle and mortar, and anthocyanins were extracted from powdered fruit tissues by addition of 1 % HCl (4 mL per 1 g tissue) and stirring. After being left overnight at room temperature, the homogenate was centrifuged at 10,000 x*g* for 10 min, and the clear supernatant was recovered. The total anthocyanin content was determined spectrophotometrically by the pH differential method, which uses the difference of absorbance at 520 and 700 nm and at pH 1.0 and 4.5 (Oki *et al.*, 2011).

Results and discussion

The average maximum and minimum temperatures during fruit growth and maturation in the three cultivation of Experiment 1 are shown in Table 1. The results are shown as the relation between fresh weight and diameter of the fruits in Fig. 1. Fruit fresh weights and diameters differed with the cultivar and cultivation experiment. There was a pseudo-parallel correlation between the fruit diameter and fresh weight. In detail, however, there was little difference in the fruit fresh weight and in fruit diameter with the cultivar in the first cultivation (22.0 g in 'Toscana Violet' and 23.4 g in 'Blue Bingo') and the third cultivation (12.5 g in 'Amakko', 13.0 g in 'Blue Bingo' and 13.6 g in 'Toscan Violet'), although the fresh weights in the first cultivation was almost double that in the third cultivation. In the second cultivation, fruit fresh weights were distributed more widely with the cultivar (8.5 g for 'Amakko', 11.5 g for 'Toscana Violet' and 17.2 g for 'Blue Bingo'). We speculated that the extremely high temperature during fruit growth and maturation during the second cultivation

Table 1. Cultivation of three tomato cultivars in Experiment 1 conducted in 2014 and 2015

Cultivar	Cultivation No.	Date		Duration of fruit growth and maturation (D) (days)	Average temperature during D (°C)	
		Anthesis of the 1st truss	End of harvest of the 3rd truss		Maximum	Minimum
	1	ND	ND	ND	ND	ND
Amakko	2	July 31	Sep. 29	61	37.7	22.2
	3	Dec. 5	Mar. 6	92	24.6	14.1
	1	May 15	July 10	57	35.2	19.2
Toscana Violet	2	July 31	Oct. 7	69	37.1	21.8
	3	Dec. 12	Mar. 23	101	24.8*	14.5*
	1	May 20	July 7	49	35.8	19.5
Blue Bingo	2	Aug. 7	Oct. 7	62	37.5	22.2
	3	Dec. 19	Mar. 16	88	24.7*	14.9*

* Until March 11, 2015, ND: Not determined

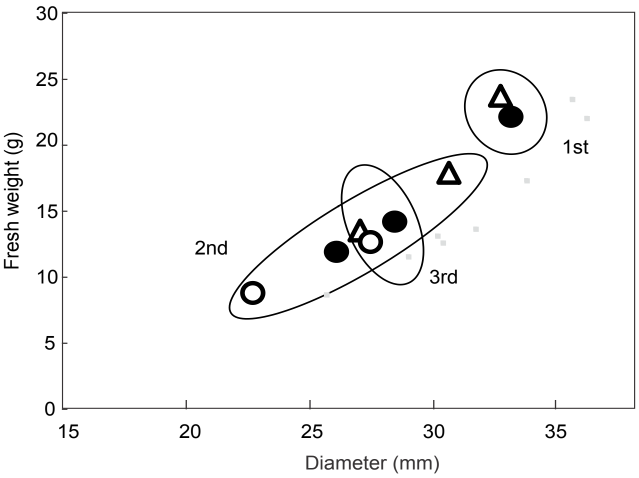

Fig.1. Relationship between fruit diameter and fresh weight. Each point is the mean of 38 to 80 fruits, except for that of 'Blue Bingo' in the 2nd cultivation, in which severe heat injury caused the decrease in the number of harvested fruits to only six. Data are for the first, second and third cultivation of Experiment 1. ○, Amakko; ●, Toscana Violet; △, Blue Bingo

affected the normal metabolism in fruits resulting in wide dispersion of fruit fresh weight (Fig. 1). Moreover, 'Blue Bingo' plants suffered injury caused by high temperature at the beginning of anthesis of the 2nd truss and thereafter, resulted in death of the growing part of the plants, in the second cultivation. Collectively, these results showed that temperature during fruit growth and maturation affect the fruit fresh weight (also fruit diameter, in other word, fruit size), and extremely high temperature would cause damage in plants.

Purple pigmentation could not be detected on the fruits of 'Toscana Violet' and 'Blue Bingo' in the first and second cultivation of Experiment 1. In these fruits, anthocyanins could not be detected from the fruits by spectrophotometric assay.

On the other hand, as shown in Fig. 2, the fruit skin of 'Toscana Violet' and 'Blue Bingo' tomatoes in the third cultivation showed clear purple pigmentation. Purple pigmentation was more vivid in 'Blue Bingo' fruits than in 'Toscana Violet' fruits. 'Amakko' tomato, the control cultivar, showed no purple pigmentation. The anthocyanin contents determined by spectrophotometry were 0.02–0.05 mg (g whole fruit)$^{-1}$ (n=9) in 'Blue Bingo' fruits, but it was undetectable in 'Toscana Violet' fruits. In 'Blue Bingo' fruits, anthocyanins were present mainly in their pericarp and adjacent mesocarp tissues.

Fig. 3 shows the pigmentation in 'Blue Bingo' fruits, which were treated at three different night-and-day temperature regimes for one week, then left at room temperature for two weeks after harvest. There was vivid purple pigmentation in the fruits grown under the night-and-day temperature regime of 15-22°C for one week, but no or faint pigmentation under those of 20-27 and 25-32°C. After being left at room temperature for one week, the fruits which had been treated with the night-and-day temperature regime of 25-32°C became red, indicating ripening of the fruits. After two weeks, the fruits which had been treated with the night-and-day temperature regime of 20-27°C, also became red. Fig. 4 shows cross-sections of 'Blue Bingo' fruits, which were treated with the night-and-day temperature regime of 15-22°C for one week (Fig. 4A) and those ripened on vine in a greenhouse. The

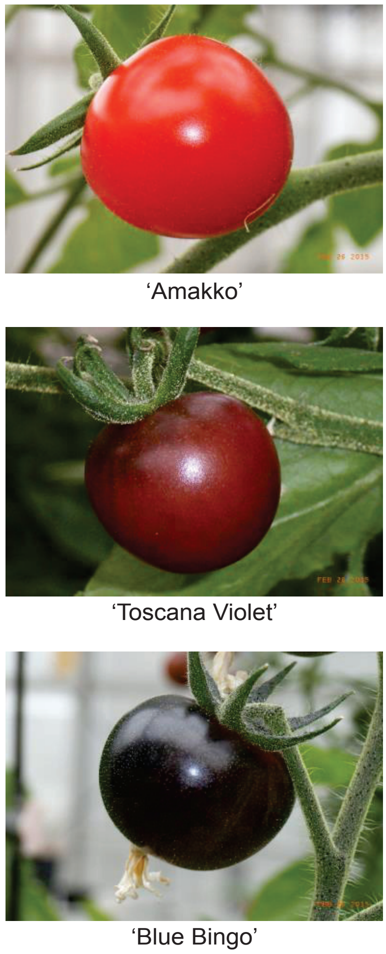

Fig. 2. Appearance of whole fruits of three cultivars in the third cultivation of Experiment 1.

former fruits accumulated anthocyanin in the skin and outer green pericarp, whereas the latter ones in the same location, though the pericarp was red. The results shown in Figs 3 and 4 suggest that accumulation of purple pigments (anthocyanins) and red pigment (lycopene) are differently regulated; *i.e.*, anthocyanin accumulation is induced by relatively low temperature, but lycopene accumulation during fruit ripening, which is in turn induced by high temperature.

The present findings suggested that anthocyanin biosynthesis in tomato fruits was suppressed at a high temperature during fruit growth and maturation. This is consistent with the finding that anthocyanin formation is stimulated at a low temperature but inhibited at a high temperature in purple rice seeds, which accumulated anthocyanin in the aleurone layer during the rice grain filling of rice kernels (Nishida, 2011).

Previously, it was reported that high light intensity is required for anthocyanin production and accumulation in purple tomato fruits, which is induced by expression of *Aft* gene (Giorgiev, 1972; Mes *et al.*, 2008; Gonzali *et al.*, 2009). On the other hand, few studies have addressed the effects of temperature on the production and accumulation of anthocyanin in purple tomato fruits. For instance, in their first report on *Aft* tomato, Jones *et al.* (2003) gave no information on temperature during cultivation in fruit expressing medium to high levels of anthocyanin. Sapir *et al.* (2008) reported that they grew *Aft* tomatoes in the open field and/or in a screen

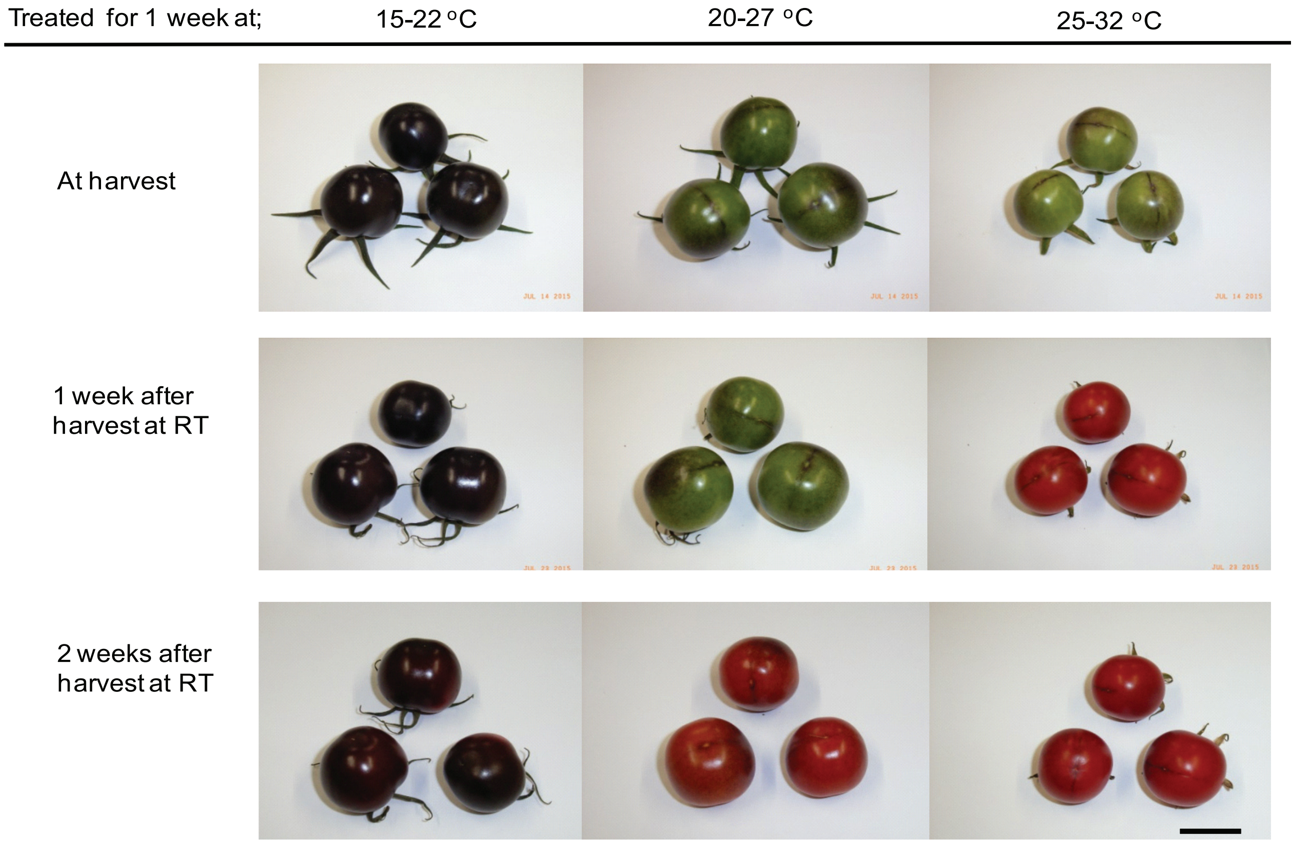

Fig. 3. Appearance of whole fruits of 'Blue Bingo', which were treated for one week at different night (8 h)-and-day (16 h) temperature regimes given at the top of the figure. Photographs were taken on the harvest day just after temperature treatment, and one and two weeks after being left at room temperature. RT means room temperature. The scale bar indicates 3 cm.

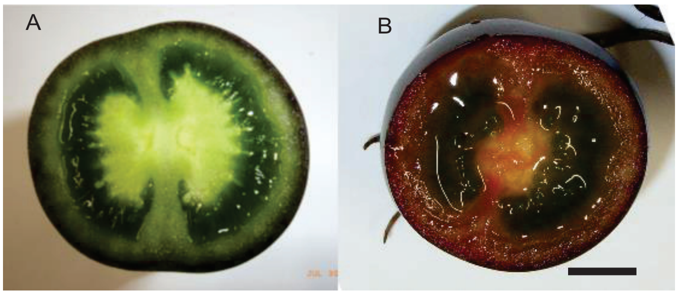

Fig. 4. Cross-sections of 'Blue Bingo' fruits, which were treated by the night-and-day temperature regime of 15-22°C for one week (A), and those ripened on vine in a glass house (B). The scale bar indicates 1 cm.

house, but did not refer to temperature during cultivation, but the minimal temperature of 18°C during the winter season. Mes *et al.* (2008) did not describe the effect of temperature on expression of anthocyanin, although they cultivated tomatoes in a greenhouse kept at 18°C at night and 25°C in the day.

In conclusion, the present study demonstrated that temperature during fruit growth and maturation is critical for expression of anthocyanins in some purple cherry tomatoes. It is suggested that purple tomato cultivation is suitable in temperature-controlled glass house and open fields under cool or moderate climate without extremely hot summer.

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