Effects of Nighttime Heating on Cell Size, Acid Invertase Activity, Sucrose Phosphate Synthase Activity, and Sugar Content of Melon Fruit

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ABSTRACT. To determine the relationship among cell size, acid invertase (AI) activity, sucrose phosphate synthase (SPS) activity, and sucrose accumulation in melon (*Cucumis melo* L.) during early development [from 6 to 16 days after anthesis (DAA)], fruit were heated at night to a minimum of 20 °C. Cells of heated fruit were larger than those of control fruit at 16 DAA but smaller at 50 DAA. AI activity was lower and SPS activity was higher in heated than in control fruit up to 26 DAA. Sucrose, glucose, and fructose contents at 26 and 50 DAA were higher in heated than in control fruit. Heating caused cells to reach mature size earlier than those of control fruit, and maturity was accompanied by earlier decline in AI activity and an earlier increase in SPS activity that promoted soluble sugar accumulation.

In Japan, melons grown in plastic film greenhouses during spring and shipped in early summer have low sucrose content. In this cultivation method, nighttime temperatures decrease to nearly 10 °C during early fruit development, and this likely accounts for the low sucrose content.

Nighttime heating of melon (Kano, 2006) or watermelon (*Citrullus lanatus* Matsum. et Nakai) during the early stages of fruit development (from 6 to 16 DAA) accelerated cell enlargement; consequently, there is high sucrose content in the fruit (Kano et al., 2008). Formation of increased numbers of larger cells in plants grown at warmer minimum temperatures during early fruit development (from anthesis to 32 DAA) was found to promote active sucrose accumulation in melon fruit (Kano and Fukuoka, 2006), and increased numbers of larger cells in Japanese pear (*Pyrus serotina* Rehd.) treated with gibberellic acid also showed increased sucrose accumulation (Kano, 2003). In contrast, restricting fruit size by treatment with succinamic acid 2,2-dimethylhydrazide (Kano, 2004) or by mechanical means (Kano, 2009) reduced the number of large cells and suppressed sucrose accumulation in melon fruit. These results suggest that when sucrose is not needed for cell growth, it is stored in larger, mature cells.

Elevated sucrose level in melon fruit is associated with a decline in acid invertase activity (Chrost and Schmitz, 1997) and an increase in SPS activity (Gao et al., 1999; Hubbard et al., 1989; Hubbard and Pharr, 1990; Lingle and Dunlap, 1987). These results suggest that declining AI activity and increasing SPS activity are criteria for mature cells. We surmise that sucrose actively accumulates in large cells, and for the purpose of this study, we consider that larger cells are mature cells. Here, we test the hypothesis that nighttime heating of melon fruit causes

early cell maturity, which, in turn, stimulates soluble sugar accumulation.

Materials and Methods

PLANT MATERIALS AND SAMPLING. ‘Earl’s Knight Soshunban-shu’ melon seeds were planted in a seed bed on 2 Mar. 2009, and nursery plants were spaced at 40-cm intervals in a plastic film greenhouse on 5 Apr. Flowers (n = 30) that opened on 10 May were divided into two groups (heated and control) and used in this experiment. Fruit were held attached and fruit in the heated group were enclosed in polyvinylchloride boxes (Fig. 1) from 6 to 16 DAA (16 to 26 May) with a minimum nighttime temperature of 20 °C. Air temperature inside and outside the fruit heating boxes was measured hourly using a thermo recorder equipped with thermistors (TR-71S; T and D, Matsumoto, Japan). Control group fruit were grown in the same plastic film greenhouse without heating boxes. Fruit (n = 5) were harvested at the end of the heat treatment, 26 and 50 DAA, and the maximum longitudinal and latitudinal lengths of each fruit (designated as fruit length and diameter, respectively) and fresh weights were measured.

Fruit were cut in half latitudinally and two 20-mm-thick cross-sections were collected for analysis: the cross-section from the maximum transverse diameter toward the peduncle end was analyzed for biochemical analyses and one from the maximum transverse diameter toward the calyx end was analyzed for cell morphology (Fig. 2). A 40-mm-wide section was collected from both cross-sections and cut into 20 × 10-mm segments (L1–L5 and R5–R1) as shown in Figure 2.

CELL MORPHOLOGY. Segments L3 and R3 from the calyx-side cross-section were analyzed for cell size and dehydrated in an ethanol series [70%, 80%, 90%, and 100% (v/v)] before being embedded in paraffin. Seven 10-μm-thick sections were prepared from each paraffin block, and the clearest section from

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Fig. 1. A schematic of using a heating apparatus to warm melon fruit. An electric heater and thermostat were set inside the apparatus.

Fig. 2. A schematic of sampling each melon fruit for analysis. Segments taken for cell morphology, enzyme and sugar analyses. An illustration of the collection of rectangular sections for cell size, enzyme and sugar analysis in melon fruit. This is an example for a fruit 50 d after anthesis.

each block was examined under a microscope. As shown in Figure 3, the maximum dimension of individual cells on the longest horizontal diameter of the segments was measured.

PREPARATIONS FOR BIOCHEMICAL ANALYSES. Segments L3 and R3 from the peduncle-side cross-section were used for enzyme analysis (one each) and for sugar analysis (one each) (Fig. 2).

Fresh melon tissue was ground in a chilled mortar using a 1:3 tissue:buffer ratio with 3-morpholinopropanesulfonic acid (Mops)-NaOH (50 mM, pH 7.5), 5 mM $MgCl_2$, 1 mM Na_2EDTA , 2.5 mM dithiothreitol, and 0.05% (v/v) polyoxyethylene-*p*-isooctylphenol (Triton X-100; Nacalai Tesque, Kyoto, Japan).

Fig. 3. An illustration of the measurement of cell size of melon fruit 16 d after anthesis. Black dots indicate the actual cells measured.

Homogenates were centrifuged at 11,000 g_n for 15 min at 4 °C and the supernatants were removed and stored at -33 °C until analysis. All chemicals were purchased from Nacalai Tesque unless otherwise indicated.

ACID INVERTASE ASSAY. AI activity was determined in 100- μ L reaction mixtures containing 20 μ L supernatant, 50 mM citrate-phosphate (pH 4.8), and 66 mM sucrose. Mixtures were incubated at 35 °C for 30 min. A 10- μ L aliquot of the reaction was ended by adding 1.5 μ L of Glucose C2 (Wako Pure Chemical Industries, Osaka, Japan), and the amount of glucose produced was measured by the Mutarotase-GOD method (Miwa et al., 1972).

SUCROSE PHOSPHATE SYNTHASE ASSAY. Two hundred microliters of supernatant was dialyzed twice for 30 min against 200 mL of 10 mM Mops-NaOH by oscillatory microdialysis system (Cosmo Bio, Tokyo, Japan) and diluted 1:8 in deionized water. SPS activity was determined in 100- μ L reaction mixtures containing 40 μ L diluted dialyzed extract, 50 mM Mops-NaOH (pH 7.5), 5 mM $MgCl_2$, 12 mM D-fructose-6-phosphate disodium salt (fructose 6-P), 12 mM D-glucose-6-phosphate dipotassium salt hydrate (glucose 6-P), and 12 mM uridine-5'-diphosphoglucose disodium salt. Mixtures were incubated at 35 °C for 30 min. The reaction was ended after 12 min with the addition of 200 μ L of 30% (w/v) KOH. After cooling, 1.8 mL of 0.14% (w/v) anthrone in 13.8 M H_2SO_4 was added and the solutions were incubated in a 40 °C water bath for 20 min. Once cooled, color development in the solutions was measured at 620 nm.

PROTEIN CONTENT ASSAY. Protein content in each extract was determined by the Bradford protein assay (Bradford, 1976) with bovine serum albumin as the standard. Briefly, a 50- μ L aliquot of each extract was assayed with this added to 2.5 mL of Coomassie protein assay kit (available on the web page), and after that, the absorbance at 595 nm was measured.

SUCROSE, GLUCOSE, AND FRUCTOSE ANALYSES. For sugar analyses, segments from the peduncle-side cross-section were wrapped in cheesecloth and squeezed using pincers to collect juice into a beaker. All procedures in this section, except boiling to inactivate enzymes, were done at less than 4 °C. The juice was heated in boiling water for 20 min to inactivate enzymes, diluted 1:10 in distilled water, and centrifuged at 8000 g_n for 15 min. The supernatant was filtered through a 0.45- μ m filter, and 10 μ L of the filtrate was applied to a high-performance liquid chromatograph (LC-10ADvp; Shimadzu, Kyoto, Japan) equipped with an

analytical column (Shim-pack SCR-101C; Shimadzu) heated to 80 °C by a column oven (CTO-10Avp; Shimadzu) and a refractive index detector (RID-10A; Shimadzu). Sampling was carried out at 0.8 mL·min⁻¹ by an autosampler (SIL-20AC; Shimadzu).

Results

FRUIT GROWTH. The minimum temperature in the heating boxes was ≈20 °C, which was almost 10 °C higher than that in the control greenhouse (Fig. 4). Fruit length, diameter, and weight did not differ significantly between heated and control fruit at any DAA (Fig. 5).

CELL SIZE. Cell size of heated fruit at 16 DAA was significantly larger ($P < 0.05$) than that of control fruit, but cell size of heated fruit at 50 DAA was significantly smaller than that of control fruit (Fig. 6). Cell size at 26 DAA did not differ between treatments. Cell growth over the 10-d periods from 16 to 26 DAA and from 26 and 50 DAA were 2.3 and 1.7 $\mu\text{m}\cdot\text{d}^{-1}$, respectively, for the heated fruit and 6.3 and 4.1 $\mu\text{m}\cdot\text{d}^{-1}$, respectively, for control fruit.

ENZYME ACTIVITY. AI activity decreased with fruit development in both treatments (Fig. 7). There was no significant difference in AI activity between treatments at any DAA resulting from high variance in the values (Fig. 7). SPS activity of heated fruit at 16 and 26 DAA was significantly higher than in control

Fig. 4. Ambient temperature in the plastic film greenhouse and in the heating box recorded every hour during the nighttime heating treatment period.

Fig. 5. Effect of nighttime heating of fruit on the growth of melon fruit. Fruit were heated during the night for 10 d from 6 to 16 d after anthesis (DAA) and collected at 16, 26, and 50 DAA. Vertical bars are SE (n = 5).

fruit (Fig. 8). SPS activity in both treatments at 50 DAA was less than 7 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ protein.

SUGAR CONTENT. Sucrose was not detected in either treatment at 16 or 26 DAA (Fig. 9). Mean glucose (6.4 g·L⁻¹) and

Fig. 6. Effect of nighttime heating of fruit on cell size in central portions of melon fruit. Fruit were heated for 10 d from 6 to 16 d after anthesis (DAA) and collected at 16, 26, and 50 DAA. *Significantly different at $P < 0.05$ by t test. Vertical bars are SE (n = 5).

Fig. 7. Effect of nighttime heating of fruit on acid invertase (AI) activity of melon fruit. Fruit were heated for 10 d from 6 to 16 d after anthesis (DAA) and collected at 16, 26, and 50 DAA. Vertical bars are SE (n = 5).

Fig. 8. Effect of nighttime heating of fruit on sucrose phosphate synthase (SPS) activity of melon fruit. Fruit were heated for 10 d from 6 to 16 d after anthesis (DAA) and collected at 16, 26, and 50 DAA. *, **Significantly different at $P < 0.05$ and 0.01 by t test. Vertical bars are SE (n = 5).

Fig. 9. Effect of nighttime heating of fruit on sugar content of melon fruit [sucrose (S), glucose (G), fructose (F)]. Fruit were heated for 10 d from 6 to 16 d after anthesis (DAA) and collected at 16, 26, and 50 DAA. *Significantly different at $P < 0.05$ by t test. Vertical bars are SE (n = 5).

fructose (26.9 g·L⁻¹) content of juice from heated fruit at 16 DAA was 24% and 53% of that of control fruit, respectively, but glucose (15.0 g·L⁻¹) and fructose (41.0 g·L⁻¹) content of juice from heated fruit at 26 DAA was greater than that of control fruit (8.5 and 34.5 g·L⁻¹, respectively). Sucrose, glucose, and fructose content at 50 DAA of juice from heated fruit was 2.0, 3.0, and 1.3 times higher, respectively, than those in control fruit (Fig. 9).

Discussion

Nighttime heating of fruit during early stages of fruit growth accelerated the growth of melon (Kano, 2006; Kano and Fukuoka, 2006) and watermelon fruit (Kano et al., 2008). However, the results shown in Figure 5 suggest that this accelerated growth did not occur in the present experiment.

In the present study, at 16 DAA, cells of fruit heated early in their development were larger than those of control fruit. Nighttime heating of fruit in early stages of fruit development has been shown to accelerate cell enlargement in melon (Kano, 2006) and watermelon fruit (Kano et al., 2008). Moreover, cell enlargement was greater in melon fruit of plants grown at higher temperatures compared with that of plants grown at lower temperatures (Kano and Fukuoka, 2006). Taken together, these results suggest that nighttime heating of melon fruit in early stages of development accelerates cell expansion, but cells of heated fruit in the present study were smaller than those of control fruit at the later stage of development (50 DAA). Cells of melon fruit heated early in development enlarged little during the later period of development, resulting in fruit that were the same size as control fruit (Kano, 2006). Cells of grape berries (*Vitis vinifera* L.) grown at higher temperatures during early development were smaller at the later stages of development than those grown at lower temperatures (Yukinaga, 1964). In the present study, cell growth rate of heated fruit was lower than that of control fruit at each stage of development. These results suggest that the growth of cells subjected to high temperatures early in fruit development is suppressed during later stages of development. Therefore, fruit heated during early stages of development mature earlier and grow little during later stages of development, but cells at the later development stage still support increased sucrose content.

SPS activity was higher in heated fruit than in control fruit at 16 and 26 DAA. In heated fruit compared with control fruit, hexose content at 16 DAA was lower, and hexose content at 26 DAA and sucrose and hexose content at 50 DAA were higher. Increases in the proportion of sucrose in melon fruit (Gao et al., 1999; Hubbard et al., 1989; Hubbard and Pharr, 1990; Lingle and Dunlap, 1987; McCollum et al., 1988; Pharr and Hubbard, 1994) and butter squash (*Cucurbita maxima* D.) at maturation (Irving et al., 1997) were associated with a decline in AI activity and an increase in SPS activity. SPS activity is important for determining the soluble sugar content of fruit of many species (Hubbard et al., 1991). These reports show that fruit maturation is accompanied by an increase in SPS activity, resulting in an increase in fruit sucrose content. Increased sucrose content of melon (Kano, 2006) and watermelon fruit (Kano et al., 2008) by nighttime heating early in development and high sucrose content in netted melon fruit grown at higher nighttime temperature (Kano and Fukuoka, 2006; Suzuki and Masuda, 1961; Suzuki et al., 1986, 1993) are considered to be results of higher temperature accelerating fruit development. Therefore, it is reason-

able to consider that higher nighttime temperature can induce an increase in SPS activity and an increase in sucrose content in heated fruit because the rate at which cells mature is accelerated. Because nighttime heating may induce earlier cell maturity, despite lower SPS activity at 50 DAA, sucrose content was higher in heated fruit at 50 DAA (Fig. 9). Lower hexose content in heated fruit at 16 DAA can be attributed to the use of hexose for cell growth and maturation during the night at higher temperatures. Hexose seems to be actively accumulated actively in heated fruit at 26 DAA because cells are nearly mature, which may explain why sucrose content is high in heated fruit.

Therefore, the following conclusions can be made regarding the relationship among cell size, SPS activity, and sucrose accumulation in melon fruit. Cells of untreated fruit mature later and they only have a short duration of active sucrose accumulation. Cells of melon fruit grown with nighttime heating during early stages of fruit development grow larger and mature early, which induces an earlier increase in SPS activity, which in turn increases the duration of sucrose accumulation, leading to high soluble sugar content in the fruit.

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