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Heating bearing shoots near fruits promotes sugar accumulation in melon fruit

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ABSTRACT

Shoots near fruits of *Cucumis melo* L. were continuously heated at night to a minimum of 30 °C from the fifth day after anthesis (DAA). Minimum and maximum ambient temperatures in the heating apparatus were about 24 and 37 °C, respectively, and these temperatures were 13 and 7 °C higher, respectively, than the minimum and maximum control ambient temperatures in the greenhouse. Minimum and maximum temperatures of fruit near heated shoots (designated heated fruit) were 15 and 28 °C; these temperatures were 3 and 2 °C higher, respectively, than those of control fruit. Flesh temperature of the heated fruit measured by thermograph was also much higher than that of the control fruit. Heated fruit weighed significantly less than control fruit. Cell size of heated fruit was smaller than that of control fruit. Sucrose phosphate synthase (SPS) activity and mean sucrose content were higher in heated fruit than in control fruit.

1. Introduction

In Japan, melons grown in plastic film greenhouses in spring have a low sugar content. Night-time temperatures in these greenhouses often reach 10 °C during early fruit development. We hypothesized that these low night-time temperatures result in the low sucrose content of the fruit. Night-time heating of melon (*Cucumis melo* L.) (Kano, 2006) or watermelon (*Citrullus lanatus*; Matsum. et Nakai) fruit (Kano et al., 2008a,b) during the early stage of fruit development (from 6 to 16 days after anthesis (DAA)) accelerates cell enlargement, and this accelerated cell enlargement leads to an increase of sucrose content. A high proportion of large cells in plants grown at higher minimum temperatures during early fruit development (from anthesis to 32 DAA) promotes active sucrose accumulation in melon fruit (Kano and Fukuoka, 2006). These results suggest that sucrose accumulates in larger cells of fruits heated during early developmental stages.

The main vascular connections in melon fruits are from the receptacle and mesocarp to the fruit axis and the seeds (Kanahama and Saito, 1986). This means that the architecture of the fruit vasculature causes inorganic nutrients in xylem and photosynthates in phloem to translocate first to the fruit axis and then to the seeds. Therefore, it may be possible to heat fruits by heating inorganic nutrients and photosynthates in the vascular bundle, rather than by heating the outside of fruit. In an attempt to increase sucrose content of the fruit, we heated shoots near melon fruit to heat the inorganic nutrients and photosynthates in the vascular bundles.

Increases in the sucrose content of melon fruit are associated with a decline in acid invertase (AI) activity (Chrost and Schmitz, 1997) and an increase in sucrose phosphate synthase (SPS) activity (Gao et al., 1999; Hubbard et al., 1989; Hubbard and Pharr, 1990; Lingle and Dunlap, 1987). These findings indicate that declining AI activity and increasing SPS activity are closely related to sucrose accumulation in melon fruit.

To test the hypothesis that heating shoots near melon fruit causes early soluble sugar accumulation, we measured temperatures, growth, cell size, sucrose metabolizing enzyme activity, and sugar content in fruit of *C. melo* L. grown with and without heated shoots.

2. Materials and methods

2.1. Plant materials and sampling

Earl's Knight Soshunbanshu (*C. melo* L.) melon seeds were planted in a seed bed on 2 March, 2010, and nursery plants were spaced at 40-cm intervals in a plastic film greenhouse on 5 April. The flowers that opened on 11 May were used in this experiment. Shoots near fruits were continuously held in a heating apparatus (Fig. 1); from 5 DAA (16 May), a minimum night-time temperature of 30 °C was maintained with an electric heater (10 cm long; LT-3, Tokyo Kogyo Boyeki Shokai Ltd.) set in a polyvinyl chloride pipe (13 cm long, 3 mm wide and 6 cm in diameter). Internal fruit temperature was measured at a point along a radius 3 cm from the

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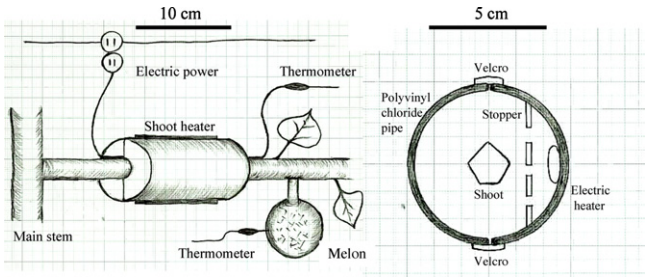

Fig. 1. Schematic diagram of the heating apparatus. An external view is shown on the left and a transverse cross-section is shown on the right.

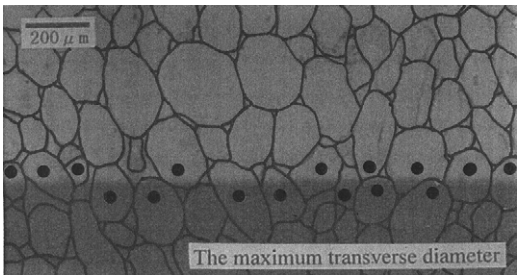

Fig. 3. Schematic diagram of the position of the cells measure to estimate cell size. The size and number of cells were measures in tissue samples taken from fruit collected 40 days after anthesis. The black dots represent the cells measured to estimate cell size.

outer surface of the fruit from 25 May to 29 May. Fruits from each treatment were collected 40 DAA, and fruit growth was investigated. Furthermore, in 2011 melon cv. Earl's Night Soshunbanshu was grown in a same way as 2010 in a plastic film greenhouse. On 1 June shoots near fruits, which set on 20 May, were continuously held in the heating apparatus to a minimum of 35 °C during night. Then fruits were collected at 11:55 pm on 1 July and immediately photos of a whole fruit and equatorial section of the fruit were taken by infrared thermography (R300W2, NEC Avio Infrared Technology Co. Ltd., Tokyo).

2.2. Tissue sampling and measurements of fruit cell size

Two disks, each approximately 20 mm thick, were excised from each fruit in each treatment group (Fig. 2); one disk, used for analysis of cell size, was cut from the maximum transverse diameter and 20 mm toward the peduncle end; and the other disk, used for analysis of sugar content and enzyme activity, was taken from the maximum transverse diameter and 20 mm toward the calyx end.

A strip that was centered on the maximum diameter of each disk and was approximately 20 mm long and 20 mm wide was removed from each disk taken from the peduncle side (Fig. 2). Rectangular parallelepipeds (designated segments), 10 mm wide, were sampled from the strip, which comprised the diameter of the disk, and segments from the central portion of the left-hand and right-hand (L2–L3 and R3–R2, respectively) of the mesocarp. Every fruit

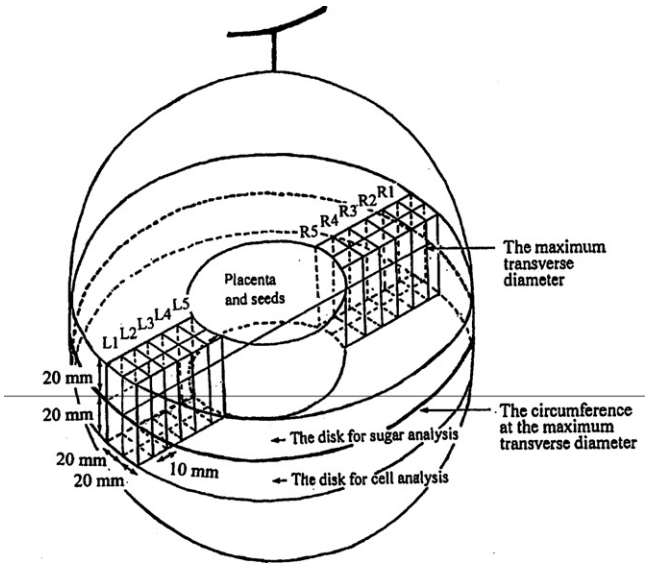

Fig. 2. Schematic diagram of the tissue sampling scheme. Parallelepiped samples of tissue were taken from mesocarp. These tissue samples were then used to measure cell size, enzyme activity, or sugar content of heated and control fruit. This diagram represents a fruit collected 50 days after anthesis.

segment collected from each treatment group was dehydrated in a series of successive ethanol solutions (70%, 80%, 90%, and 100% (v/v, ethanol:water)) before being embedded in paraffin. Sections ($n = 7$; each 10 μm thick) were prepared from these paraffin blocks, and the clearest section from each segment from each treatment group was examined under a microscope (hereafter referred to as segments L1–5 or R1–5). The maximum diameter of individual cells on the maximum transverse diameter of each segment was measured (Fig. 3).

2.3. Tissue sampling for analysis of enzyme activity and sugar content

A strip that was centered on the maximum diameter of each disk and was 40 mm wide was removed from each disk taken from the calyx side. From the central portion of the left-hand and right-hand mesocarp of the strip, each rectangular parallelepiped (designated as a segment) 10 mm wide was sampled across the diameter of the disk. Each 40 mm × 10 mm strip was cut into two strips along the maximum diameter line; one strip was used for enzyme analysis the other was used to measure sugar content. From the 20 mm × 10 mm strip for enzyme analysis the central rectangular parallelepiped (designated as a segment) 10 mm wide portion of the left-hand (L2 and L3) and right-hand (R3 and R2) mesocarp of the strip were sampled across the diameter of the disk. AI and SPS activities were measured in L2, L3, R3, and R2 and a mean value from all four samples was calculated.

2.4. Enzyme extraction

Melon tissue was ground in a chilled mortar using a 1:3 tissue:buffer ratio. The buffer contained 50 mM Mops-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM Na_2 EDTA, 2.5 mM DTT, and 0.05%, v/v Triton X-100. Homogenates were centrifuged at 11,000 rpm for 15 min at 4 °C, and the supernatant from each sample was stored at −33 °C.

2.5. Acid invertase (AI) assay

Reaction mixtures (100 μl) used to measure AI activity contained 50 mM citrate–phosphate (pH 4.8), 66 mM sucrose, water, and 20 μl sample supernatant. Reaction mixtures were incubated at 35 °C for 30 min. Addition of 1.5 μl of Glucose C2 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to 100 μl of the mixture terminated the AI reaction, and production of glucose was measured using the Mutarotase-GOD method (Miwa et al., 1972).

2.6. Sucrose phosphate synthase (SPS) assay

The supernatant was dialyzed before determination of SPS activity. Reaction mixtures (100 μl) used to determine SPS activity