Sucrose Accumulation and Related Metabolizing Enzyme Activities in Seeded and Induced Parthenocarpic Muskmelons

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ABSTRACT. To clarify the cause of low sucrose accumulation in seedless ‘Crest Earl’s’ netted muskmelon [*Cucumis melo* L. (Reticulatus Group)] fruit induced by CPPU, the activity level of sucrose metabolizing enzymes was compared between seeded and seedless fruit. CPPU promoted growth of the ovary in both pollinated and nonpollinated flowers until 10 days after anthesis (DAA), and thereafter the growth rate of nonpollinated fruit was lower than in the controls. Sucrose accumulation of seedless fruit remained lower than in seeded fruit, but there was no difference in fructose and glucose content between seeded and seedless fruit. Acid invertase activity declined sharply 20 DAA in seeded and seedless fruit, and was hardly detectable at 35 DAA, when sucrose accumulation began. Neutral invertase (NI) activity in both seeded and seedless fruit decreased from 20 DAA until 35 DAA; thereafter, NI activity in seeded fruit remained relatively constant, with a small but insignificant increase in maturity. Sucrose synthase (SS-c: sucrose cleavage direction) activity in seeded fruit decreased from 20 to 30 DAA, and then increased as fruit matured, while SS-c activity in seedless fruit did not change during development. Sucrose phosphate synthase (SPS) activity in seeded fruit increased from 25 to 30 DAA and remained relatively constant until harvest. SPS activity in seedless fruit declined gradually from 30 to 45 DAA, then remained at a low level. Sucrose synthase (SS-s: sucrose synthesis direction) activity in seeded fruit increased rapidly after 30 DAA, concomitant with sucrose accumulation. In contrast, SS-s activity in seedless fruit increased only slightly after 30 DAA indicating levels of SS-s activity are closely related to sucrose accumulation in parthenocarpic seedless muskmelons. Chemical name used: [1-(2-chloro-4-pyridyl)-3-phenylurea] (CPPU).

Muskmelon [*Cucumis melo* (Reticulatus Group)] fruit sometimes fail to be fertilized, and consequently to set fruit, particularly under unfavorable conditions for pollination and fertilization, such as low temperature and low solar radiation (Hayata et al., 2000b; Jones, 1965). When [1-(2-chloro-4-pyridyl)-3-phenylurea] (CPPU), a fruit set accelerator, was applied to nonfertilized ovaries, this treatment often resulted in parthenocarpic seedless fruit.

It has been reported that sugar content of parthenocarpic fruit is lower than that of seeded fruit in loquat (*Eriobotrya japonica* L.) (Tani et al., 1990), sweet cherry (*Prunus avium* L.) (Taira et al., 1990), and grape (*Vitis* L. sp.) (Kondo and Kawai, 1998). Consequently, using CPPU to produce seedless muskmelons would lead to problems with fruit quality due to reduced sugar concentration (Hayata et al., 2000b; Tanakamaru, 1989). We reported in a previous study that the decline in sugar content of parthenocarpic fruit is due to a decrease in sucrose accumulation, because sucrose is a major storage sugar in muskmelons (Hayata et al., 2000b). Many studies have shown that sucrose synthase (EC 2.4.1.13) acting toward sucrose synthesis (SS-s) plays an important role in sucrose accumulation in sucrose-accumulating fruit, such as peach (*Prunus persica* Batsch var. *vulgaris*) (Kobashi et al., 1999; Moriguchi et al., 1990; Moriguchi et al., 1991), Japanese pear (*Pyrus serotina* Rehder var. *culta*) (Moriguchi et al., 1992), and loquat (Bantog et al., 1999), whereas sucrose phosphate synthase [SPS, (EC 2.4.1.14)] is the key enzyme involved in sucrose accumulation in strawberry (*Fragaria × ananassa* Duch.) (Hubbard et al., 1991), wild tomato

(*Lycopersicon hirsutum* Humb) (Dali et al., 1992; Miron and Schaffer, 1991), buttercup squash (*Cucurbita pepo* L.) (Irving et al., 1997), and banana (*Musa sapientum* L.) (Cordenunsi and Lajolo, 1995; Hubbard et al., 1990). McCollum et al. (1988) and Schaffer et al. (1987) suggested that sucrose accumulation in muskmelons is associated with an increase in SS-s activity accompanied by a decrease in acid invertase [AI, (EC 3.2.1.26)] activity. In contrast, Lingle and Dunlap (1987) reported that SPS activity increased with a decrease in AI activity. Moreover, Hubbard et al. (1989) compared levels of enzyme activity between two muskmelon genotypes with different sucrose accumulation, and found that SPS, rather than SS, was the key enzyme in sucrose accumulation. Thus, to clarify the cause of reduced sucrose content in seedless muskmelons, this study compared activities of sucrose metabolizing enzymes between hand-pollinated seeded fruit and seedless fruit induced by 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU), in a single genotype.

Materials and Methods

PLANT MATERIAL AND CULTURE. ‘Crest Earl’s’ netted muskmelons were grown in beds in a greenhouse under natural photoperiod and irradiation at Hiroshima Prefectural University from 20 Apr. through 19 Aug. 1998. Greenhouse air temperatures were maintained between 18 and 28 °C by a heating system (NEPON KA-201; Nepon LTD, Hofu, Japan). Soil in the bed was a sandy loam with 2% organic matter and pH 6.3. Irrigation in the beds was automatically controlled by an irrigation system regulated by a tensiometer (DM-8P Melon; Takemura Electric Works LTD, Tokyo, Japan). Water potential in the soil (pF: the logarithm of a difference in the chemical potential between pure water and soil water) was maintained at pF 1.8 until rapid fruit growth stage [until 25 d after anthesis (DAA)], pF 2.0 from net formation stage until 7 d before harvest (51 DAA), then pF 2.4 until harvest (58

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DAA). Fertilizer was applied twice, the first application was a preplant broadcast application of 900 kg·ha⁻¹ of 14N-14P-36K and the second application was a sidedress application of 326 kg·ha⁻¹ of 46 N at anthesis. The vines were trained vertically and topped at the 23rd node. All lateral shoots were cut above the second node leaving only lateral shoots at the 13th to 15th nodes. Female flowers on the first node of the lateral shoots were used for the experiment. At 10 DAA, fruit were thinned to leave one fruit per plant.

Three treatments were applied as follows: (P) female flowers were hand-pollinated at anthesis without any other treatments, (PC) female flowers were hand-pollinated and the ovaries were sprayed with a solution of CPPU at 20 mg·L⁻¹ at anthesis, and (EC) the ovaries of nonpollinated female flowers of which the corolla was covered with a paper bag the day before anthesis were sprayed with a solution of CPPU at 20 mg·L⁻¹ at anthesis. The paper bags were removed 3 DAA. One hundred plants were placed randomly in each of three treatments. Fresh weight (FW) of individual fruit was recorded, and fruit tissue was sampled from the mesocarp for analysis of sugar content and enzyme activity.

ANALYSIS OF SOLUBLE SUGAR CONTENTS. Samples of mesocarp tissue (5 g) were homogenized in 20 mL 80% (v/v) hot ethanol (70 °C) with a blender (ULTRA-TURRAX T25; Janke & Kunkel GMBH Co., Staufen, Germany). The homogenate was boiled for 30 min and centrifuged at 10,000 g_n for 5 min at room temperature. The residue was suspended in 20 mL hot ethanol and the procedure was repeated two times. Supernatants were pooled, passed through a 0.45-μm membrane filter (Millipore Co., Bedford, Mass.), concentrated in vacuo, and dissolved in distilled water. Sugars in the solution were separated by a high-performance liquid chromatography (Shimadzu Co., Kyoto, Japan) with a Shodex Asahipak NH2P-50 4E column (Showadenko Co., Tokyo) at 40 °C using 65% acetonitrile solution (1 mL·min⁻¹) as a mobile phase. The separated sugars were detected with a refractive index detector (model 133 RI; Gilson Medical Electronics, Inc., Middleton, Wis.) identified by a comparison of their retention times with those of authentic sugars.

ENZYME EXTRACTION. To extract enzymes, the method of Hubbard et al. (1989) was adopted. All procedures were conducted at 4 °C. A 2-g sample of mesocarp was homogenized in 8 mL of 50 mM Mops-NaOH buffer (pH 7.5) containing 1 mM Na₂EDTA, 5 mM MgCl₂, 2.5 mM dithiothreitol (DTT), bovine serum albumin (BSA) at 0.5 mg·mL⁻¹, and 0.05% (v/v) Triton X-100. The homogenate was centrifuged at 20,000 g_n for 5 min. The supernatants were desalted on Sephadex G-25 (PD-10) columns equilibrated with 50 mM Mops-NaOH buffer (pH 7.5), 5 mM MgCl₂, 2.5 mM DTT, and BSA at 0.5 mg·mL⁻¹.

ACID AND NEUTRAL INVERTASE ASSAYS. Reaction mixtures (100 μL) to determine AI activity contained 50 mM sodium acetate buffer (pH 4.8), 66 mM sucrose, and 50 μL desalted extract. Reaction mixtures were incubated at 35 °C and terminated at 30 min by addition of alkaline CuSO₄ Somogi-Nelson reagent. A desalted extract boiled for 2 min was used as a blank. Hexose sugar concentration was determined by the Somogi-Nelson procedure (Nelson, 1944). The assay to determine neutral invertase (NI) activity was the same as for AI except the reaction mixtures contained 50 mM Mops-NaOH buffer (pH 7.5) instead of 50 mM sodium acetate buffer.

SPS AND SUCROSE SYNTHASE ASSAYS. Reaction mixtures (100 μL) to determine SPS activity contained 50 mM Mops-NaOH buffer (pH 7.5), 5 mM NaF, 15 mM MgCl₂, 25 mM glucose 6-P, 12 mM fructose 6-P, 12 mM UDPG, and 50 μL desalted extract. Reaction mixtures were incubated at 35 °C and terminated at 30 min with 200 μL of 30% KOH. A desalted extract boiled 2 min was used as a blank. Sucrose production was determined by the anthrone reagent. The procedure for SS assay in sucrose synthesis direction (SS-s) was identical to that of SPS except the reaction mixtures contained 12 mM fructose and did not contain NaF, glucose 6-P, or fructose 6-P. The procedure for SS assay in the sucrose cleavage direction (SS-c) was identical to that of NI except the reaction mixtures contained 6 mM UDP. SS-c activity was estimated by subtracting the respective neutral invertase activity for the same sample.

STATISTICAL ANALYSIS. Means and SE values were calculated based on the data obtained from 10 replications for FW and 5 replications for sugar and enzyme analysis. Significant differences between the seeded fruit (P or PC treatments) and seedless fruit (EC treatment) in SS-s and SPS activities at each DAA were detected by *t* test. Significance of coefficients of determination (*r*²) of linear regression analysis between sucrose content and SS-s or SPS activities was detected at *P* ≤ 0.01 or 0.001 using the means of P, PC, or EC treatment at each DAA, respectively.

Results

CPPU applied to ovaries at anthesis promoted growth in both pollinated and nonpollinated flowers until 15 DAA (Fig. 1). Thereafter, FW of nonpollinated fruit with CPPU treatment was lower than that in the pollination-only treatment (treatment P) at every sampling time. At harvest, the seedless fruit were ≈80% the weight of the treatment P fruit.

Sucrose content of fruit in the treatment P and in the pollination + CPPU treatment (treatment PC) increased rapidly from 30 to 50 DAA, then slowed until harvest (Fig. 2A). On the other hand, sucrose in seedless fruit in the nonpollination + CPPU treatment (treatment EC) also increased from 30 to 50 DAA, but the rate of increase was lower than in treatments P and PC; between 50 DAA and harvest, the sucrose content did not increase, resulting in

Fig. 1. Changes in fresh weight (FW) of seeded and seedless 'Crest Earl's' netted muskmelons induced by CPPU treatment. Vertical bars = SE (n = 10).

about half the amount (3.5%) compared with the control (6.9%). There were no clear differences in sucrose content between the P and PC treatments. Differences in glucose and fructose content among the three treatments were nonsignificant at all stages, ranging between 1.4% and 2.6% (Fig. 2B and C).

Activity levels of AI and NI in mesocarp showed a similar change during fruit development among the three treatments: the levels were highest at 20 DAA and declined until 35 DAA, when sucrose accumulation began (Fig. 3). After 25 DAA, NI activity for treatment C was slightly lower than for the other treatments (Fig. 3B). In seeded fruit, SS-c activity decreased from 20 DAA then increased gradually after 30 or 35 DAA (Fig. 3C). However, in seedless fruit, this activity remained lower than in seeded fruit during most of the period of fruit development. SS-s activity in seeded fruit increased rapidly from 30 DAA, concomitant with sucrose accumulation (Fig. 4A). In contrast, SS-s activity in seedless fruit increased slightly from 30 DAA, and was significantly lower at $P < 0.01$ ($n = 5$) than in seeded fruit. Therefore, SS-s activities have a close positive correlation ($r^2 = 0.847$, $n = 24$, $P < 0.001$) with the sucrose content of muskmelons. SPS activity in seeded fruit increased slightly from 25 DAA, whereas in seedless fruit it declined gradually (Fig. 4B). As a result, the level of activity was significantly lower than in seeded fruit during sucrose accumulation.

Discussion

There was no difference between treatment P and treatment PC in either sugar accumulation or the level of activity of sucrose metabolizing enzymes during later growth stages. CPPU induced parthenocarpy in nonpollinated fruit, but it did not affect accumulation of sucrose or the level of enzyme activity in pollinated seeded fruit. Therefore, the relationship between sugar content

and the activity level of sucrose metabolizing enzymes is discussed based on a comparison of seeded and seedless muskmelons.

It has been reported that sugar content is low in seedless fruit induced by plant growth regulators, such as loquat (Tani et al., 1990) and sweet cherry (Taira et al., 1990). The sugar content of seeded grapes exceeded that of parthenocarpic seedless grapes when the harvest time was extended (Kondo and Kawai, 1998). We also observed previously that sucrose accumulation is low in parthenocarpic muskmelons induced by CPPU (Hayata et al., 2000b), which coincides with findings of the present study. To clarify the cause of low sucrose accumulation in seedless muskmelons, we compared levels of activity of sucrose metabolizing enzymes in both seeded and induced seedless fruit. AI activity declined 20 DAA and was barely detected during sucrose accumulation. This loss of AI activity has been demonstrated in many studies (Hayata et al., 2000a; Hubbard et al., 1989; Lingle and Dunlap, 1987; McCollum et al., 1988; Schaffer et al., 1987) and is related to sucrose accumulation in fruit. Sucrose cleavage enzymes are generally thought to increase sink activity related to sucrose translocation (Hatch et al., 1963; Hubbard and Pharr, 1992; Lowell et al., 1989; Sung et al., 1994). In the present study, the sink strength of seeded muskmelons is considered to be higher than that of seedless fruit. This difference in sink strength might be related to the observation that levels of activity of NI and SS-c were higher in seeded fruit than in seedless fruit. These results suggest that those sucrose cleavage enzymes are playing some role in regulation of sink activity in the fruit.

McCollum et al. (1988) and Schaffer et al. (1987) reported that SS-s plays an important role in sucrose accumulation in muskmelons, whereas Lingle and Dunlap (1987) demonstrated

Fig. 2. Changes in (A) sucrose, (B) fructose, and (C) glucose contents of seeded and seedless 'Crest Earl's' netted muskmelons induced by CPPU treatment. Legend in (A) applies to all figures. Vertical bars = SE ($n = 5$).

Fig. 3. Changes in (A) acid invertase (AI), (B) neutral invertase (NI), and (C) sucrose synthase (SS-c) (cleave direction) activities of seeded and seedless 'Crest Earl's' netted muskmelons induced by CPPU treatment. Vertical bars = SE ($n = 5$).

Fig. 4. Changes in (A) sucrose synthase (SS-s) (synthesis direction) and (B) sucrose phosphate synthase (SPS) activities of seeded and seedless 'Crest Earl's' netted muskmelons induced by CPPU treatment. Vertical bars = SE (n = 5).

that SPS, not SS, is associated with sucrose accumulation. However, Hubbard et al. (1989) pointed out that reported SS and SPS activity levels in those earlier studies were too low to determine their roles in sucrose accumulation, and that there were problems with the extraction procedures. They also suggested that SPS is the key enzyme, based on an observation that SPS activity in the fruit of a sweet genotype of muskmelon rose rapidly from 6 to 32 $\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}\text{FW}$ concomitant with sucrose accumulation, while the SPS activity in a nonsweet genotype increased more slowly. We observed a significant difference in the level of SPS activity between seeded ($\approx 20 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}\text{FW}$) and seedless fruit ($\approx 6.0 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}\text{FW}$) during sucrose accumulation. These results support the importance of SPS in sucrose accumulation indicated by Hubbard et al. (1989).

SS-s activity increased from the point when sucrose accumulation began in muskmelons under all three treatments. SS-s activity in seeded fruit with high sucrose content increased significantly, and SS-s activity in seedless fruit with low sucrose content showed a gradual increase. As a result, there was a highly significant correlation between sucrose accumulation and SS-s activity. SPS has been known to be a key enzyme in sucrose accumulation in strawberry (Hubbard et al., 1991), wild tomato (Dali et al., 1992; Miron and Schaffer, 1991), buttercup squash (Irving et al., 1997), banana (Cordenunsi and Lajolo, 1995; Hubbard et al., 1990), and melon (Hubbard et al., 1989; Lingle and Dunlap, 1987), all of which store sucrose. On the other hand, SS-s activity is also known to be associated with sucrose accumulation in peach (Kobashi et al., 1999; Moriguchi et al., 1990, 1991) and loquat (Bantog et al., 1999). Our results indicate that changes in the level of both SPS and SS-s activity are closely associated with the change in sucrose accumulation. We suspect that SS-s plays a very important role in sucrose accumulation, based on comparison of activity levels of sucrose metabolizing enzymes in one genotype of melon fruit in two situations: one

with low sucrose accumulation in induced parthenocarpic fruit, and the other where sucrose accumulation normally increases in seeded fruit.

The main role of SS is considered to cleave sucrose to supply UDP-glucose (Amor et al., 1995; Chourey and Nelson, 1976), although SS catalyzes sucrose metabolism in both the synthesis and cleavage directions. Recently, Suzuki et al. (1996) found two different SS isozymes in Japanese pear (*Pyrus serotina* Rehder var. *culta* Rehder); one strongly catalyzed toward sucrose cleavage and the other toward sucrose synthesis. We observed very high SS-c activity levels and low levels of SS-s activity at an early stage of muskmelon development (Hayata et al., 1999). Moreover, a decline in SS-c was observed with a rapid increase in SS-s at the middle and later stages of fruit development while sucrose was accumulating. These observations demonstrate there may be SS isozymes of different capacities in melon fruit which warrant future study. We conclude that the low AI activity, SS-s, and SPS all play important roles in accumulation of sucrose in muskmelons, and that low levels of SS-s and SPS activity are mainly responsible for the decrease in sucrose content in seedless fruit.

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