Changes in Sugar Content and Sucrose Synthase Enzymes during Fruit Growth in Eggplant (*Solanum melongena* L.) Grown on Different Polyethylene Mulches

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Abstract. This study clarified the role of the key enzymes [sucrose phosphate synthase (SPS), sucrose synthase (SS)] in the sucrose synthesis of eggplant (*Solanum melongena* L.) cultured on different polyethylene mulches. Sugar concentration in fruit and SPS and SS activities in leaf and fruit were measured during the different fruit development stages. Total sugar concentration was the highest on the reflective mulches throughout all the developmental stages of eggplant and followed by the white, the black, and transparent mulches. The concentrations of all three individual sugars (sucrose, glucose, and fructose) increased during fruit development on all four polyethylene mulches. Although no specific pattern in enzyme activities of leaf and fruit related to the sugar accumulation of eggplant fruit was observed, the reflective and white mulches triggered higher sucrose synthase enzyme activities than did the black and transparent mulches.

Eggplant (*Solanum melongena* L.), as a member of the Solanaceae family, mostly demands high temperature and light during its growth (Krug, 1991; Messiaen, 1989). It is usually grown in open fields during summer and in greenhouses during winter. In particular, lack of light in cultivating eggplant may result in poor fruit quality (Sezgin, 2007). Therefore, different reflective film mulches are usually used to enhance lighting. Black, white, and reflective film mulches in vegetable crops can improve their yield as well as quality (Costello, 1995).

Depending on the mulch color and the local climatic conditions, microenvironments around plants can be altered to improve crop growth and yield (Decoteau et al., 1990). Dark-colored films increase soil temperature through greater heat absorbance. Lighter colors, however, reflect more sunlight, minimize changes in soil temperature, and increase light intensity around the plant canopy (Orzolek and Murphy, 1993). Reflective mulches can induce higher rates of photosynthesis by increasing the intensity of

selected active light wavelengths in the lower canopy depending on the plant density (Porter and Eitzell, 1982). Black and white film mulches are widely used to manipulate soil temperature. They have been found to aid plant growth with diverse climatic conditions (Albregts and Chandler, 1992; Brown et al., 1992; Porter and Eitzell, 1982).

Fruit growth requires an increase in sink activity, which is provided by activating carbohydrate metabolism. Sucrose synthase (SS) and sucrose phosphate synthase (SPS) are enzymes that affect sucrose metabolism and that are associated with the developmental processes of the sink organ (Balibrea et al., 1996; Islam et al., 1996; Kim et al., 2003; Sung et al., 1994). The application of sugar-induced activity of SS and changes in the concentration of sucrose in the sink organ are known to control SS activity (Claussen et al., 1986). These findings suggest that SS activity is regulated by sucrose. This study, therefore, was carried out to clarify the distribution of sugars and related sucrose enzymes at four developmental stages of the eggplant fruit cultured on different polyethylene mulches.

Materials and Methods

Plant material and growing conditions. Japanese-type eggplant (*Solanum melongena* L. cv. Chikuyou) seedlings were planted onto

beds mulched with black, white, reflective (silverized film), and transparent (control) films (0.1 mm thick) as polyethylene mulches. The beds consisted of three rows 9 m long and 110 cm wide. The rate of light reflection was 42% to 48% for the reflective film, 7% to 8% for the black film, 16% to 19% for the white film, and 11% to 12% for the transparent film. The seedlings were grown in a greenhouse at a soil temperature of 25/20 °C day/night. Planting density was 80 cm; only plants from the central rows were harvested for analysis. Each experiment was designed as a randomized complete block with three replicates.

Sugar analysis. To analyze the sugar content, fruit samples were collected at different development stages on the basis of fruit length, ranging from 2 to 3 cm to 18 to 20 cm in length. Samples of fruit tissue (10 g) were macerated in a mortar with acid-purified sand and 80% ethanol. After centrifugation, residues were extracted and centrifuged twice using 30 mL ethanol at 12,000 × g for 10 min. The extracts were filtered and evaporated; the total volume of each sample was adjusted to 10 mL with distilled water. Sugar (sucrose, glucose, and fructose) composition and concentration of samples were analyzed using high-performance liquid chromatography. Before injection, 5 mL of the sample was passed through C₁₈ Sep-Pak cartridge (Waters, Milford, MA) and then further filtered through 0.45 µm Acrodisc prefilter (Pall Corporation, USA). The sugars were eluted onto a Waters Sugar-Pak™ I column (6.5 × 300 mm) isocratically with a mobile phase of water at a flow rate of 0.5 mL·min⁻¹. The eluate was monitored using a refractive index detector. Each experiment was conducted in triplicate and involved combined extracts of three different eggplant fruits.

Enzyme assays. To investigate the activity of sucrose synthase enzymes, fruit or leaf samples were collected at different development stages on the basis of fruit length, ranging from 2 to 3 cm to 18 to 20 cm in length. SS and SPS were extracted following procedures of Song and Ko (1997). Samples of fresh leaf (2 g) and freeze-dried fruit tissue (0.5 g) were homogenized in a prechilled mortar and pestle along with 10 mL of buffered solution containing 0.4 M Hepes [N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid)]/KOH buffer (pH 7.5), 20 mM MgCl₂, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM ethylenediaminetetraacetic acid, 20% (v/v) glycerol, 2% (v/v) ethylene glycol, 20 mM mercaptoethanol, 20 µL aprotinin, and 3% (w/v) polyvinylpyrrolidone. The resulting mixture was centrifuged for 5 min at 13,000 × g, filtered through glasswool, and centrifuged again for 15 min at 25,000 × g. A 2 mL adjust of supernatant was desalted by passage through a Sephadex G-25 column pre-equilibrated with 20 mM Hepes/KOH buffer (pH 7.2), 10 mM MgCl₂, 0.1 mM PMSF, 10% (v/v) glycerol, and 5 mM mercaptoethanol. Enzymes were extracted at 4 °C. SPS reactions were initiated by adding 80 µL of desalted plant extract to a reaction mixture containing 7.7 mM fructose-6-P,

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19.3 mM MgCl₂, 28.2 mM glucose-6-P, 7.1 mM UDP-glucose (uridine 5'-diphosphoglucose), and 62 mM Hepes/KOH (pH 7.5) in a total volume of 240 µL as described by Salvucci et al. (1990).

For SS, the reaction consisted of 10 mM fructose, 5 mM MgCl₂, 2 mM UDP-glucose, 100 mM Hepes/KOH (pH 7.2), and 80 µL plant extract with a final volume of 1 mL. All reactions were carried out at 30 °C. At specific times, reactions were ended by boiling 60-µL aliquots for 2 min and centrifuged at 13,000 × g for 2 min. The reaction mixtures of 50 µL were incubated with shaking by addition of 100 µL 30% (w/v) KOH and placing the tubes in a boiling water bath for 10 min. After cooling, 3 mL anthrone was added (Hubbard et al., 1989), and the mixtures were incubated at 40 °C for 15 min. After cooling, color development was measured at 620 nm and quantified using a standard calibration curve with standards. Sucrose concentration was measured based on the anthrone method of Van Handel (1968). UDP was measured using the method described by Salvucci et al. (1990). Preliminary research indicated that the SS activity in the synthetic direction at pH 7.2 was equivalent to the breakdown activity at an optimum 6.5 pH. To make it simple, SS was therefore assayed in the synthetic direction. Protein was determined using the Bio-Rad Protein Kit (Bio-Rad Laboratories, Hercules, CA) through the Coomassie blue method of Bradford (1976). Each experiment was conducted in triplicate and consisted of the combined extracts of three eggplant fruits.

Data analysis. Statistical analysis was performed using procedures of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). When the means were significant at the F-test, the means were separated by a least significant difference test.

Results and Discussion

Sugar analysis. The concentrations of sucrose, glucose, and fructose in eggplant fruits were measured. Glucose and fructose concentrations were higher than that of sucrose. Glucose and fructose are predominant reducing sugars, but their contents vary with light intensity. Total or individual sugar concentrations were higher in the reflective and white mulches at all developmental stages than the black and transparent mulches. The concentration of each sugar in plants of the four polyethylene mulch treatments increased during all fruit development stages (Fig. 1).

The results suggest that amount of light reflection in the reflective film was the highest and that its light intensity can be uniformly distributed under the plant canopy. This can confirm that difference in sugar composition depends on light intensity variations as affected by different polyethylene mulches. These findings, therefore, suggest that increase in light intensity stimulates the sugar content of eggplant fruits. This study demonstrates that the reflective polyethylene mulches significantly increased sugar concentration. Singh (1992)

Fig. 1. Changes in contents of sucrose, glucose, and fructose in eggplant fruits during fruit growth with different polyethylene mulches.

discussed the effects of mulching on eggplant, in which the amount of light plays a role in the activities of sucrose-metabolizing enzymes during fruit growth. However, the mechanism by which light stimulates sucrose synthesis of the eggplant fruit is not fully understood.

Enzyme assay. Tables 1 and 2 show the activities of the sucrose-metabolizing enzymes extracted from the leaf and fruit segments. SPS and SS activities increased during the initial fruit development stage and then decreased gradually. Significant differences ($P < 0.05$) in SPS and SS activities among development stages were observed. The reflective film mulch showed the greatest enzyme activity and followed by the white, the black, and transparent mulches. At the four mulching treatments, the activities of SPS and SS are associated with sugar concentrations at the fruit development stages (Table 1; Fig. 1).

SPS and SS activities have been established as the major mechanisms for sucrose synthesis (Huber and Huber, 1996; Stiff and Sonnewald, 1995) and determinants of sink

strength (Ho, 1988; Sung et al., 1989). A significant difference in the levels of SPS activity was observed in all treatments during fruit development. Although similar patterns to fruits were observed in leaves, the level of SPS activity was higher in fruit than in leaves of eggplant regardless of development stages or mulches. Throughout all the fruit development stages, SS activity also was consistently higher at the reflective mulch than any other mulch. SS and SPS enzymes affect sucrose metabolism and are associated with developmental processes of the sink organ. In eggplant cultivation, lighting deficiency and low temperature are believed to produce low-quality fruit (Sezgin, 2007). The difference in SS activity is notable, because it indicates higher sink strength during the stages of sugar accumulation. Photosynthetic assimilates produced in leaf chloroplasts are primarily converted into sucrose and starch. Starch accumulated during the photosynthetic period is usually mobilized in the ensuing dark period and can be converted into sucrose for export to other parts of the

Table 1. Activity of sucrose synthase enzymes in eggplant fruits from plants grown on different mulching materials.

		Developmental stage (fruit length)				
		2–3 cm	5–6 cm	12–15 cm	18–20 cm	LSD (0.05)
Enzyme	Mulches	Enzyme activity (µg sucrose/min/mg dry weight)				
SPS	Reflective	1.59 ^z	2.68	2.77	1.85	0.58
	White	1.31	2.56	2.61	1.51	0.49
	Black	1.22	2.48	2.43	1.37	0.33
	Transparent	1.11	2.53	2.35	1.45	0.35
	LSD (0.05) ^y	0.29	NS ^x	0.32	0.40	
SS	Reflective	1.68	2.62	2.48	2.28	0.56
	White	1.50	2.60	2.39	2.18	0.65
	Black	1.52	2.42	2.24	1.66	0.62
	Transparent	1.35	2.28	2.01	1.59	0.51
	LSD (0.05)	0.33	0.16	NS	0.61	

^zValues are the mean of three replicates.

^yMeans were separated by LSD (least significant difference) test ($P < 0.05$).

^xNS = nonsignificant.

SPS = sucrose phosphate synthase; SS = sucrose synthase.

Table 2. Activity of sucrose synthase enzymes in eggplant leaves from plants grown on different mulching materials.

		Developmental stage (fruit length)				
		2–3 cm	5–6 cm	12–15 cm	18–20 cm	LSD (0.05)
Enzyme	Mulches	Enzyme activity (µg sucrose/min/mg fresh weight)				
SPS	Reflective	0.48 ^z	0.56	0.52	0.51	NS
	White	0.39	0.52	0.49	0.47	0.07
	Black	0.33	0.44	0.50	0.41	0.10
	Transparent	0.35	0.53	0.49	0.43	0.09
	LSD (0.05) ^y	0.07	NS ^x	NS	0.06	
SS	Reflective	0.30	0.47	0.46	0.44	0.06
	White	0.28	0.41	0.42	0.39	0.11
	Black	0.31	0.40	0.36	0.35	0.08
	Transparent	0.26	0.45	0.33	0.40	0.09
	LSD (0.05)	0.06	NS	0.11	0.10	

^zValues are the mean of three replicates.

^yMeans were separated by LSD (least significant difference) test ($P < 0.05$).

NS = nonsignificant.

SPS = sucrose phosphate synthase; SS = sucrose synthase.

plant. Sucrose synthesized in the leaf is loaded onto the phloem sap and partitioned to other parts of the plant through the vesicular system. In sucrose-importing organs, the regulation of sucrose cleavage within the cells serves as the biochemical marker of sink strength (Ho, 1988; Sung et al., 1989). In this study, the change in SPS and SS activities were slightly associated with fruit growth (Tables 1 and 2). The regulation of SS activity may be less important compared with SPS activity in inducing fruit growth. Although both SS and SPS are sucrose synthetic enzymes, their activities appear to vary among fruit species. In pineapple and papaya fruits, SPS is actively involved in sucrose synthesis, whereas SS is known to be involved in sucrose cleavage rather than synthesis (Chen and Paull, 2000; Zhou and Paull, 2001).

This study determines the possible causes of unequal sugar distribution in eggplant fruits by examining the allocation of enzymes as the determinant of sink strength and sucrose synthesis. Sink strength at the enzyme level refers to the series of enzymatic reactions associated with sucrose storage or its interconversion. Sugar concentration in eggplant fruit was studied with focus on the regulation of sucrose and sink strength. For eggplants cultured with high canopy density, the uniform lighting stimulated by reflective mulch affects the

activities of sucrose-metabolizing enzymes. Although this study defines the possible advantages of sucrose-metabolizing enzymes by using different mulching materials in eggplant culture, it clearly showed the benefits derived from using reflective mulch in eggplant. Additional work is recommended to clarify the range of benefits and modes of action associated with different mulching materials.

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