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Sucrose Phosphate Synthase, Sucrose Synthase, and Invertase Activities in Developing Fruit of *Lycopersicon esculentum* Mill. and the Sucrose Accumulating *Lycopersicon hirsutum* Humb. and Bonpl.[1]

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ABSTRACT

The green-fruited *Lycopersicon hirsutum* Humb. and Bonpl. accumulated sucrose to concentrations of about 118 micromoles per gram fresh weight during the final stages of development. In comparison, *Lycopersicon esculentum* Mill. cultivars contained less than 15 micromoles per gram fresh weight of sucrose at the ripe stage. Glucose and fructose levels remained relatively constant throughout development in *L. hirsutum* at 22 to 50 micromoles per gram fresh weight each. Starch content was low even at early stages of development, and declined further with development. Soluble acid invertase (EC 3.2.1.26) activity declined concomitant with the rise in sucrose content. Acid invertase activity, which was solubilized in 1 molar NaCl (presumably cell-wall bound), remained constant throughout development (about 3 micromoles of reducing sugars (per gram fresh weight) per hour. Sucrose phosphate synthase (EC 2.4.1.14) activity was present at about 5 micromoles of sucrose (per gram fresh weight) per hour even at early stages of development, and increased sharply to about 40 micromoles of sucrose (per gram fresh weight) per hour at the final stages of development studied, parallel to the rise in sucrose content. In comparison, sucrose phosphate synthase activity in *L. esculentum* remained low throughout development. The possible roles of the sucrose metabolizing enzymes in determining sucrose accumulation are discussed.

The green fruited, wild *Lycopersicon* species (subspecies *Eriolycopersicon*) has been reported to contain high levels of sucrose, as compared with the red-fruited (*Eulycopersicon*) species, which contains primarily glucose and fructose in the mature fruit, with only trace amounts of sucrose (4). The species that accumulate sucrose are characterized by high TSS[2] content (°Brix) and are of potential importance in improving fruit quality of cultivated *Lycopersicon esculentum* Mill. (20). Sucrose-accumulating storage tissues are characterized by a metabolic transition during development. During the initial growth phase, soluble acid invertase activity was relatively high, declining concomitantly with sucrose accumulation. Such a transition has been reported, for example, in sugar beet (7), sugar cane (8), sweet melon (12, 16, 18, 21), carrot (19), and citrus (14). In Solanaceae other than *Lycopersicon*, we have observed such a transition in *Solanum muricatum* Ait. (pepino) fruit (22). Among the *Lycopersicon* spp., Manning and Maw (17) reported that the sucrose-accumulating *Lycopersicon peruvianum* Mill. and *Lycopersicon hirsutum* had low soluble invertase activity, as compared with *L. esculentum*. Recently Yelle *et al.* (26) reported a loss of acid invertase activity during the development of the sucrose-accumulating *Lycopersicon chmielewskii*.

In some of the above storage organs, sucrose accumulation is accompanied by an increase in sucrose synthase activity (6, 16, 18, 21, 22), suggesting that this enzyme may play a physiologically significant role. However, such a rise in activity has not been reported consistently (12).

A rise in SPS activity parallels the rise in sucrose during the development of sweet melon (12, 16). Recently Hubbard *et al.* (12), following a procedure that minimized loss of labile SPS activity during tissue preparation and extraction, reported a sharp increase in SPS activity in sweet melons, reaching an activity of 32 μmol of sucrose (g fresh weight)$^{-1}$ h^{-1}. They suggested that sucrose accumulation is determined by the balance between sucrose synthesis (SPS activity) and degradation (invertase and sucrose synthase activities). Recently Fieuw and Willenbrink (6) also reported SPS activity, previously undetected in sugar beet root, using refined assay conditions.

In this paper, we report a sharp rise in SPS activity, together with the loss of soluble acid invertase, during the development of *L. hirsutum*, a green-fruited, sucrose-accumulating species. The roles of sucrose-metabolizing enzymes in determining sink strength and sucrose accumulation are discussed.

MATERIALS AND METHODS

Plant Material

Plants of *Lycopersicon hirsutum* Humb. and Bonpl. LA 1777 (kindly supplied by Dr. M. Pilowsky and originally a generous gift to him from Dr. C. M. Rick) and commercial

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[2] Abbreviations: TSS, total soluble solids; SPS, sucrose phosphate synthase; daa, days after anthesis.

cv and breeding lines of *Lycopersicon esculentum* Mill. were grown in 15-L pots in a greenhouse, according to standard methods. Flowers of *L. hirsutum* were sib-pollinated and tagged on the day of anthesis, whereas flowers of *L. esculentum* were allowed to self-pollinate and were tagged on the day of anthesis.

Sampling for TSS, Carbohydrate, and Enzyme Assays

Portions of the whole harvested fruit were used for determination of TSS content, using an Atago N-1 hand-held refractometer. Portions (about 500 mg fresh weight) were immediately placed in 80% EtOH for sugar and starch determination. An additional sample was frozen at −20°C for assay of invertase activity. A separate sample was immediately frozen in liquid nitrogen and stored at −78°C for SPS and sucrose synthase assays.

Carbohydrate Determination

Soluble sugars were extracted three times in hot EtOH, and starch content was measured in the insoluble fraction after treatment with amyloglucosidase, as described previously (21). Soluble sugars were separated by HPLC using a Bio-Rad Fast Carbohydrate Column with double-distilled H_2O as solvent, according to the manufacturer's directions, and refractometric detection. Sucrose, glucose, and fructose were identified by their retention times and quantified according to standards.

Enzyme Extraction and Assays

Acid Invertase

Invertase activity was extracted and assayed according to the method of Schaffer *et al.* (22) with modifications. Approximately 1 g fresh weight frozen tissue was homogenized in a Kinematica homogenizer in 3 volumes of extraction buffer containing 50 mM Hepes-NaOH (pH 7.5), 0.5 mM Na-EDTA, 2.5 mM DTT, 3 mM diethyldithiocarbamic acid, 0.5% (w/v) BSA, and 1% (w/v) insoluble PVP. PVP was necessary during the extraction of *L. hirsutum* since extracts quickly browned in its absence (1). After centrifugation at 18,000*g* for 30 min, supernatants were dialyzed for about 16 h against 25 mM Hepes-NaOH (pH 7.5) and 0.25 mM Na-EDTA and used as the crude soluble enzyme extract. The insoluble pellet was homogenized twice in 10 mL of the extraction buffer and after centrifugation was suspended in 3 mL of 50 mM Hepes-NaOH (pH 7.5) and 0.5 mM Na-EDTA. To solubilize the 'insoluble' invertase activity, NaCl (0.2–1.0 M final concentration) was added to the initial extraction buffer.

Invertase activity was assayed in 0.6 mL of 0.1 M K_2HPO_4–0.1 M citrate buffer (pH 5), 0.2 mL 0.1 M sucrose, and 0.2 mL of enzyme extract. Incubation was for 30 min at 37°C. The reaction was stopped and reducing sugars were measured using dinitrosalicylic acid (23). Enzyme was added after the 30-min incubation for blank control. Reaction mixtures with insoluble enzyme suspensions were centrifuged before spectrophotometric reading.

Sucrose Phosphate Synthase and Sucrose Synthase

One gram of frozen tissue kept in liquid nitrogen was ground in a cold mortar in 3 mL of buffer containing 50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM Na-EDTA, 2.5 mM DTT, 0.5 mg mL^{-1} BSA, and 0.05% (v/v) Triton X-100 according to the method of Hubbard *et al.* (12), with slight modifications. A 1-mL portion of the homogenate was desalted directly by centrifugal filtration (11) at 1°C on 5-mL Sephadex G-25 columns, equilibrated with the above buffer without EDTA and Triton X-100.

SPS was determined in reaction mixtures (70 μL) containing 50 mM Hepes-NaOH (pH 7.5), 15 mM $MgCl_2$, 25 mM Fru6P, 25 mM Glc6P, 25 mM UDPGlc, and 40 μL of extract. Mixtures were incubated for 30 min at 37°C, and incubation was terminated with the addition of 70 μL of 30% KOH. Enzyme blanks were terminated with KOH at 0 min. Sucrose was assayed according to the modified anthrone method of Van Handel (24). Sucrose synthase was assayed as above but with 25 mM Fru instead of Fru6P, and in the absence of Glc6P.

RESULTS

Because green-fruited *L. hirsutum* do not change color with maturity, fruits were sampled until they were soft (70 daa). Sucrose was present in low amounts (about 15 μmol [g fresh weight]$^{-1}$) during the early stages of development, but its level rose to about 118 μmol (g fresh weight)$^{-1}$ during the period from 50 to 70 daa (Fig. 1). Glucose and fructose levels did not change significantly throughout development. Starch levels were low even at the early stages (Fig. 1) and declined even further during development.

A comparison with a number of *L. esculentum* cv and breeding lines (Table I) showed that in the mature, ripe fruit the distinguishing characteristic of *L. hirsutum* is its accumulation of high concentrations of sucrose. Glucose and fructose levels of *L. hirsutum* were similar to those in the large-fruited *L. esculentum* cv. The small-fruited *L. esculentum* cv had higher glucose and fructose concentrations than did the wild species. However, the high level of total sugars in the wild species reflects primarily the elevated sucrose content.

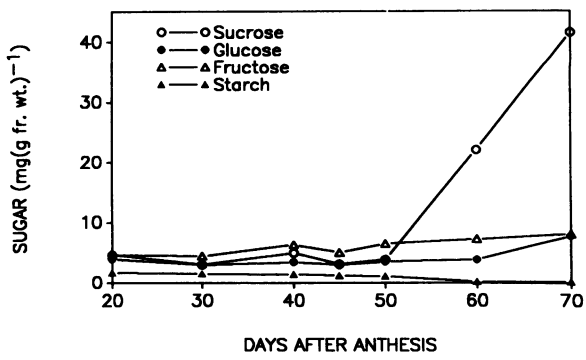

Figure 1. Sugar and starch contents of *L. hirsutum* fruit during development. Each point represents the mean of five values.

Table I. *Sugar Content of L. hirsutum and L. esculentum cv and Breeding Lines*

Line	Sugar Content				TSS	Sucrose/Hexose
	Sucrose	Glucose	Fructose	Total		
	mg (g fresh wt)$^{-1}$				*°Brix*	
L. hirstum						
LA 1777	41.2	7.8	8.2	57.2	>10.0	2.57
L. esculentum						
Large-fruited						
H_2	1.2	7.3	11.4	19.9	4.5	0.06
Summit	1.3	9.0	12.0	22.3	5.0	0.06
M-82	1.1	10.8	12.4	24.3	5.0	0.05
Small-fruited						
BR-124	2.3	14.6	21.8	38.7	6.8	0.06
CBL	2.2	15.7	17.1	35.0	7.8	0.07

Invertase activity could be separated into nonsaline buffer-soluble ('soluble') and saline buffer-soluble (presumably cell-wall bound) activities (Fig. 2). At low NaCl concentrations, there is significant acid invertase activity associated with the particulate fraction, whereas at high NaCl concentrations this activity is solubilized.

Soluble acid invertase activity is practically undetectable in 70 daa *L. hirsutum*, whereas in comparison, ripe *L. esculentum* cv had extremely high activity (Fig. 3). At early stages of development, 'soluble' acid invertase activity was present in the wild species, but this activity declined concomitantly with sucrose accumulation (Fig. 4). The insoluble acid invertase activity remained relatively constant throughout development, so that at the final stages of development practically all of the invertase activity was particulate-associated. In *L. esculentum* cv BR-124, invertase activity was present in green, immature fruit and increased sharply during the ripening process (Fig. 5). Analogous results were observed during the development of cv F-121 and breeding line P-10 (data not presented), similar to previous reports of ripening-associated increase in invertase (17).

SPS activity was measured using the precautions recently reported by Hubbard *et al.* (12). Figure 6 shows that SPS activity in 70 daa *L. hirsutum* was more than 3.5-fold higher than observed in *L. esculentum* cv that included both small-fruited and large-fruited types. Low activity was observed during early developmental stages of *L. hirsutum* fruit (Fig. 7). Activity rose sharply, parallel to sucrose accumulation, and reached an activity of 30 μmol of sucrose (g fresh weight)$^{-1}$ h^{-1} in the absence of Glc6P and 42 μmol of sucrose (g fresh weight)$^{-1}$ h^{-1} in the presence of 25 mM Glc6P. In comparison, activity levels of SPS did not change drastically during the maturation and ripening of *L. esculentum* cv BR-124, a small-fruited type (Fig. 8). Sucrose synthase activity was barely detectable in *L. hirsutum* from 40 daa (Fig. 7).

DISCUSSION

The present results show that the wild species *L. hirsutum* can be classified as a sucrose-accumulating fruit, with a biphasic pattern of sucrose accumulation, similar to sweet melon (12, 16, 18, 21), pepino (22), and *L. chmielewskii* (26). The wide spectrum of *L. esculentum* cv that we and others (4) have studied shows that this species does not accumulate sucrose to significant amounts (>30 μmol [g fresh weight]$^{-1}$).

The loss of soluble acid invertase activity during the sucrose accumulating stage, together with the relatively low activity at the early stage of development, is in agreement with previously published studies of other sucrose-accumulating *Lycopersicon* spp. (17, 26). Analogous results showing a correlation between loss of soluble acid invertase activity and sucrose accumulation have been reported in other species (8, 14, 16, 17, 19, 21, 22). It is likely to be a universal phenom-

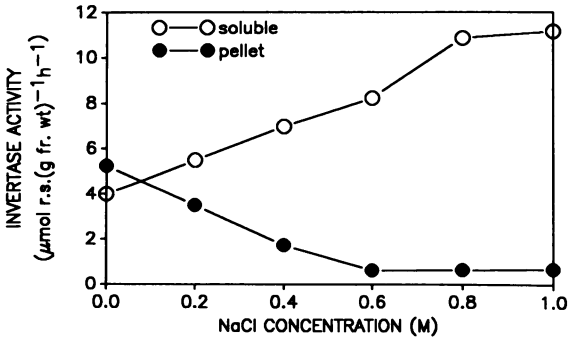

Figure 2. Effect of NaCl concentration in the extraction buffer on solubility of invertase activity in 30 daa *L. hirsutum* fruit. Each point represents the mean of two values.

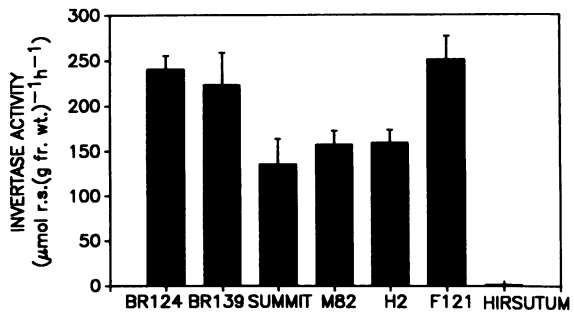

Figure 3. Levels of soluble invertase activity in ripe fruit of various cv and breeding lines of *L. esculentum* and *L. hirsutum*. Each point represents the mean of two values ± SE.

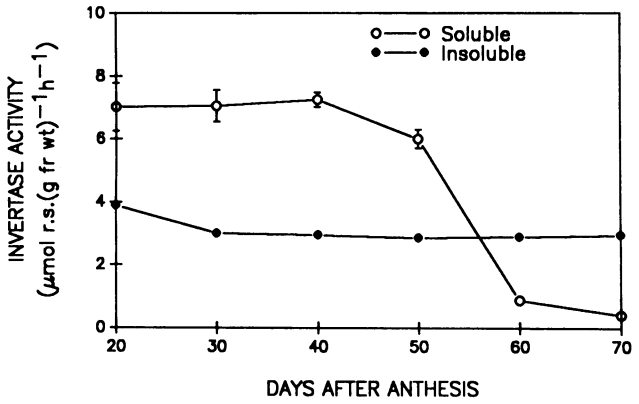

Figure 4. Levels of soluble and insoluble (pellet-associated in 0 mM NaCl extraction buffer) invertase in *L. hirsutum* fruit throughout development. Each point represents the mean of five values ± SE.

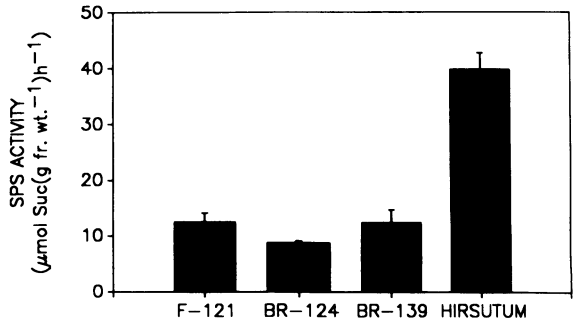

Figure 6. Levels of SPS activity in mature fruit of *L. hirsutum* and *L. esculentum* cvs F-121, BR-124, and BR-139. Each point represents the mean of three values ± SE.

enon in plant storage organs, presumably due to compartmentation of both sucrose and soluble acid invertase in the vacuole (15).

To the best of our knowledge, this is the first report of SPS activity in the *Lycopersicon* genus. The sharp rise in activity coinciding with sucrose accumulation, together with the high levels of observed *in vitro* activity, strongly suggest physiological significance with respect to the accumulation of sucrose. SPS activity has been reported in mature, sucrose-accumulating sugar cane stalks (9), sugar beet roots (6), and sweet melon fruit (12, 16). It is not unlikely that the presence of high SPS activity is characteristic of sucrose-accumulating tissues and that reports of insignificant or low activity in such tissue (16) may be due to the assay methods used. Recently some of the precautions necessary for the assay of labile SPS have been emphasized (12).

Hubbard *et al.* (12) have presented a scheme whereby sucrose accumulation in melon fruit is determined by the relationship between sucrose synthesizing (SPS) activity and sucrose breakdown (invertase and sucrose synthase) activities. Accordingly, the loss of soluble acid invertase activity prevents sucrose hydrolysis and allows the accumulation of sucrose. However, the loss of invertase activity alone would not ensure an increase in sucrose or total sugar content, and for this, high SPS activity may be necessary.

In this regard, we have observed in segregating populations of sucrose accumulating and nonaccumulating genotypes that high-acid invertase genotypes do not accumulate sucrose. However, low-acid invertase genotypes do not necessarily accumulate sucrose, implying that low-acid invertase activity allows for, but alone is not sufficient for sucrose accumulation (D Miron and AA Schaffer, unpublished results; Y Burger and AA Schaffer, unpublished results).

If SPS is indeed necessary for sucrose accumulation, this would imply a physiological-metabolic pathway that would presumably include hydrolysis of imported sucrose and subsequent resynthesis. The constant activity of insoluble acid invertase we observed could account for apoplastic hydrolysis, maintaining a sucrose gradient at the site of phloem unloading. Accordingly, the rise in SPS activity could increase the rate of sucrose accumulation by maintaining the hexose gradient between the apoplast and the cytosol, and, as such, SPS may play a role in determining sink strength. The transport of hexose sugars into the cytoplasm, sucrose resynthesis, and

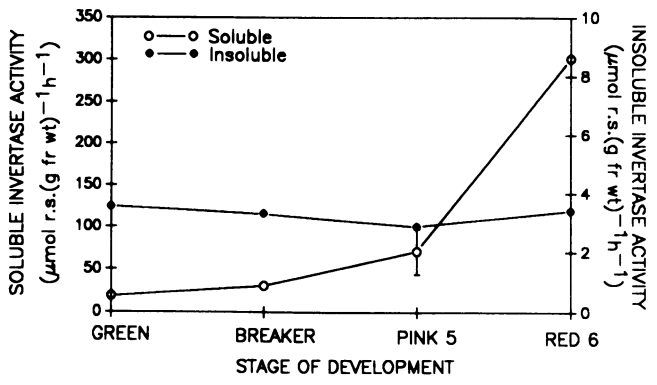

Figure 5. Levels of soluble and insoluble (pellet-associated in 0 mM NaCl extraction buffer) invertase in *L. esculentum* fruit cv BR-124 throughout development. Each point represents the mean of five values ± SE.

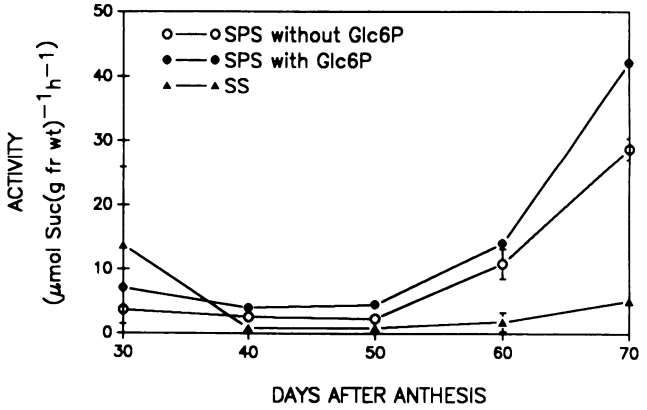

Figure 7. Levels of SPS and sucrose synthase (SS) activity in *L. hirsutum* fruit throughout development. SPS was measured in the presence or absence of 25 mM Glc6P in the assay medium. Each point represents the mean of three values ± SE.

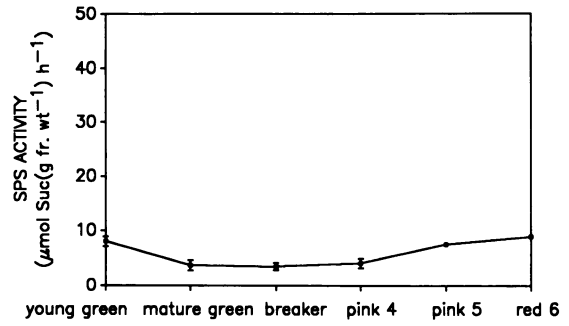

Figure 8. Levels of SPS activity of *L. esculentum* cv BR-124 at different stages of development. The red stage was reached at approximately 80 daa. Each point represents the mean of three values ± SE.

vacuolar compartmentation could be analogous to that reported for sugar cane (9, 10). Previous studies with tomato pericarp discs (3, 13) indicate that the uptake of sugars from the apoplast in *L. esculentum* is not an energy-dependent process and that the concentration gradient is most likely the driving force of movement through the fruit.

Although Yelle *et al.* (26) have suggested, based on randomization studies, that sucrose is taken up intact by *L. chmielewskii* fruit without prior hydrolysis, their conclusion was based on a study of young (20 daa) fruit, before the onset of significant sucrose accumulation. We hypothesize that during the sucrose accumulation phase, when SPS activity is high and soluble acid invertase activity is low, sucrose accumulation may occur via a dual mechanism: (a) the intact storage of translocated sucrose, without hydrolysis; and (b) the resynthesis of sucrose via SPS. At early stages of development, when SPS activity is low, the first mechanism may work alone.

In addition, the high SPS activity we observed also suggests the possibility that sucrose may be synthesized from alternative substrates other than those derived from sucrose hydrolysis. For example, nonphotosynthetic CO_2 fixation and refixation of CO_2 from fruit respiration (2) may ultimately provide substrates for SPS activity. Furthermore, fruit-derived photosynthate may also provide precursors for sucrose synthesis, and in this respect it may be important to emphasize that those *Lycopersicon* species that accumulate high levels of sucrose are green-fruited during the stage of sucrose accumulation.

The results of this study also indicate that acid invertase is not always an indication of sink strength, as pointed out previously by Yelle *et al.* (26). Although a correlation may yet be found between sink strength and invertase activity in *L. esculentum* (25), in the sucrose-accumulating *Lycopersicon* species this is not the case (26). Similarly, the correlation between starch content in the young fruit and TSS in the mature *L. esculentum* reported by Dinar and Stevens (5) does not apply to the wild species studied (Fig. 1).

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