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Sink Metabolism in Tomato Fruit[1]

III. ANALYSIS OF CARBOHYDRATE ASSIMILATION IN A WILD SPECIES

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ABSTRACT

Carbohydrate composition and key enzymes involved in carbohydrate metabolism were assayed throughout development of *Lycopersicon esculentum* and *L. chmielewskii* fruit. Translocation and assimilation of asymmetric sucrose and total soluble solids content was also determined in both species. The data showed that *L. chmielewskii* accumulated less starch than *L. esculentum*, and this was related to a lower level of ADPglucose pyrophosphorylase and a higher level of phosphorylase in *L. chmielewskii*. *L. chmielewskii* accumulated sucrose throughout fruit development rather than glucose and fructose which were accumulated by *L. esculentum*. A low level of invertase and nondetectable levels of sucrose synthase were associated with the high level of sucrose in *L. chmielewskii*. Translocation and assimilation of asymmetrically labeled sucrose indicated that sucrose accumulated in *L. chmielewskii* fruit was imported and stored directly in the fruit without intervening metabolism along the translocation path. In contrast, the relatively low level of radioactive sucrose found in *L. esculentum* fruit appeared to arise from hydrolysis and resynthesis of sucrose. The possible relationship between the level of soluble solids and differences in carbohydrate metabolism in sink tissue of the two species is discussed.

The harvestable yield of tomato appears to be regulated by the net assimilation rate of the crop, the rate of import into individual fruit, and sink activity (9). High sink demand can significantly increase the quality of the tomato fruit by high accumulation of soluble solids, an important factor for processing tomatoes. Sugars are the major components of the soluble solids content in tomato comprising approximately 65% of the soluble solids.

As reported by Gifford and Evans (6), the processes localized in sink tissue largely determine the distribution of photoassimilate between competing sinks. According to Walker and Ho (16), sink strength of a tomato fruit is principally affected by the sink activity of the fruit. The major mechanisms involved in sink activity are: (a) unloading of sucrose by the phloem, (b) hydrolysis and uptake of sugars, (c) biosynthesis and storage of carbohydrate (10). It has been suggested (16) that invertase activity may play a major role in regulating the rate of carbon translocation in tomato fruit.

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Fruit of several undomesticated tomato species have been reported to have relatively high soluble solids levels (13). Indeed, one of these species, *Lycopersicon chmielewskii*, has a soluble solids content exceeding 10%, and this species has served as a parent in the development of high soluble solids tomato varieties (13). In this paper, we have compared sink processes in *L. chmielewskii* and *Lycopersicon esculentum* to identify biochemical processes that may be involved in promoting photosynthate import into tomato fruit.

MATERIALS AND METHODS

Plant Material. *Lycopersicon esculentum*, cv UC82B, and *Lycopersicon chmielewskii*, LA 1028, were seeded in growing beds and transplanted 5 weeks later into pots. Greenhouse day and night temperatures were maintained at a minimum of 20 and 17°C, respectively, with a ventilation temperature of 24°C. Fifteen plants of each cultivar were selected and trusses were tagged at anthesis. Fruit were harvested every 7 d for 64 d and frozen at −70°C for the determination of sugar levels and enzyme activities.

Starch and Sugar Determinations and Enzyme Assays. Tissue from the whole fruit, 10 g, was ground with a tissue homogenizer in 10 ml of homogenization buffer for approximately 20 s. The homogenization buffer contained 50 mM Hepes-KOH (pH 8.3), 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EGTA, and 2 mM DTT. After homogenization, aliquots were saved for determining starch and sugar levels (using HPLC) and enzyme activities according to Robinson *et al.* (14).

Translocation of Asymmetrically Labeled Sucrose. *L. esculentum* and *L. chmielewskii* were seeded on beds in a greenhouse and grown as outlined above. Loading of leaves with asymmetrically labeled sucrose, [^{3}H]-(fructosyl)-sucrose, was performed on three plants from each cultivar with 20 d fruit on the first truss. Twenty-four h before loading, the plants were pruned of all leaves except the one above the first truss. Two fruits were kept on the first truss for *L. esculentum* while four were kept for *L. chmielewskii* because of the smaller size of the fruit. To improve sucrose penetration, approximately 1 cm^2 of leaf was abraded with carborundum between the midvein and lateral veins. A silicon well was formed around the abraded area and radioisotope solution containing 0.1 mM $MgSO_4$, 0.1 mM KCl, 0.5 mM $CaCl_2$, 5 mM Mes (pH 6), and 10 μCi of [^{3}H]-(fructosyl)-sucrose (10.1 Ci/nmol) in 100 μl was placed in the silicon well. A cover slip was then pressed over the circular well to seal it and prevent the solution from evaporating (1).

Twelve h later, fruits were harvested. Discs of tomato pericarp tissue were obtained by cutting slices 2 mm thick and 5 mm in diameter. The discs were washed for 20 s in water and 2 discs of each fruit were placed in a 20 ml vial containing 10 ml of aerated cold water to elute the apoplastic sugar. After incubation for 6 min the discs were removed and extracted in ethanol for analysis of symplastic sugar as described above. The 10 ml of water

containing the apoplastic sugars was evaporated, resuspended in 200 μl of water, and analyzed by HPLC. The sucrose peaks from both the symplast and apoplast (approximately 1.5 ml) were collected. The sucrose was enzymically hydrolyzed with 0.23 mg of yeast invertase in citrate buffer (pH 4.6) for 1 h at 37°C. After 1 h the sample was dried, resuspended in 200 μl of water, and injected into an HPLC. Sucrose, glucose, and fructose fractions were collected and counted, and the ratio of [^{3}H]glucose/[^{3}H] fructose was calculated.

Total Soluble Solids Content. Total soluble solids content was determined as °Brix with a table top model ABBE-3L Bausch and Lomb refractometer.

RESULTS AND DISCUSSION

Total Soluble Solids. Varieties of *L. esculentum* have been reported to have fruit soluble solids content of between 4 and 6% of the fruit fresh weight. The variety used in this study, UC82B, has been widely used commercially and has a relatively low soluble solids content of 5 °Brix (data not shown). In contrast, *L. chmielewskii* (LA 1028) has a high soluble solids content of 10.2 °Brix (data not shown). Because sugars are the major component of tomato fruit soluble solids, we examined the accumulation of carbohydrate and levels of carbohydrate metabolizing enzymes in both species throughout fruit development.

Starch Accumulation. In both *L. esculentum* and *L. chmielewskii*, starch accumulates in young fruit reaching a peak approximately 20 d after anthesis and then decreasing to near zero at fruit maturity (Fig. 1A). Davies and Cocking (2) observed a similar pattern of transient starch accumulation in different cultivars of tomato fruits. Between 14 and 49 d, *L. esculentum* had a significantly higher level of starch than *L. chmielewskii*.

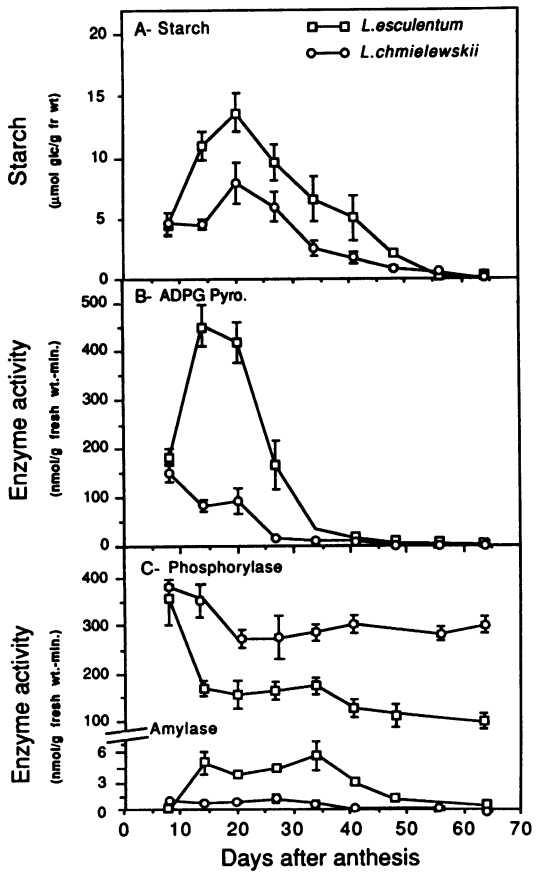

FIG. 1. Levels of starch (A), ADPG pyrophosphorylase (B), phosphorylase and amylase (C) throughout development of *L. esculentum* and *L. chmielewskii* fruit. Each point represents the mean of three values ± SE.

A positive correlation has been reported between the rate of starch accumulation and the rate of fruit growth (10). It has also been shown that a high soluble solids content at maturity is associated with an accumulation of starch early in fruit development and with a high import rate (3). However, our results show that the accumulation of starch does not seem to be associated with the high soluble solids content of *L. chmielewskii*. Recently, Ehret and Ho (4) found no correlation between starch content and dry weight accumulation in tomato fruit.

Robinson *et al.* (14) reported that levels of ADPG[5] pyrophosphorylase rather than starch degradative enzymes appeared to regulate the transient accumulation of starch in *L. esculentum*. To determine if a similar mechanism regulated starch accumulation in *L. chmielewskii* we assayed levels of ADPG pyrophosphorylase, amylase, and phosphorylase (Fig. 1, B and C). In both species the depletion of starch was associated with a decrease in ADPG pyrophosphorylase levels (Fig. 1B). Over the same period of starch degradation, amylase and phosphorylase activities remained constant or decreased slightly (Fig. 1C) suggesting that starch biosynthesis rather than degradative capacity regulated the transient accumulation of starch. *L. chmielewskii* fruits possessed higher levels of phosphorylase and lower levels of ADPG pyrophosphorylase relative to the *L. esculentum* fruit. In both species, the activity of amylase is negligible as compared to phosphorylase. The higher level of phosphorylase and lower level of ADPG pyrophosphorylase acting together may contribute to the lower level of starch in *L. chmielewskii*.

Sugar Accumulation. As *L. esculentum* fruits developed, an increase in both fructose and glucose was observed with a particularly dramatic increase between 10 and 30 d after anthesis (Fig. 2). The highest levels of both sugars were present 60 d after anthesis. *L. chmielewskii* accumulated much less hexoses than *L. esculentum*. However, the level of sucrose rose appreciably during the maturation of *L. chmielewskii* fruit to reach a maximum at 64 d after anthesis. *L. esculentum* fruit have a very low level of sucrose throughout development. Expressed as glucose equivalent units *L. chmielewskii* accumulated approximately twice the amount of soluble sugar as *L. esculentum* 60 d after anthesis.

Two enzymes involved in sucrose breakdown were studied, sucrose synthase and acid invertase. The activity of invertase was very low in the fruit of *L. chmielewskii*, decreasing as the fruit matured (Fig. 3A). In contrast, invertase activity was much higher in *L. esculentum*, rising throughout development to reach a peak 40 d after anthesis and then declining in activity. Nakagawa *et al.* (12) have also found a decline in the invertase activity in senescent tomato fruits, whereas Manning and Maw (11) have found a constantly increasing activity as fruit ripen. This discrepancy in invertase levels over development is most likely due to extraction of whole fruit as opposed to pericarp tissue. The rise of invertase activity for *L. esculentum* is associated with an increase in tissue concentration of hexoses, and the very low level of hexoses in *L. chmielewskii* may be attributed, at least in part, to the low invertase activity compared to that in *L. esculentum*. Walker and Thornley (15) suggested that metabolism of sucrose by invertase contributed to the maintenance of high rates of carbon import. If the high soluble solids content of *L. chmielewskii* is attributable to high import rates, as has been suggested for high soluble solids *L. esculentum* varieties (8), these high rates of import are obviously not associated with high invertase levels in *L. chmielewskii*.

Sucrose synthase is an alternative enzyme capable of degrading sucrose to UDPglucose and fructose (7). As we have previously

[5] Abbreviation: ADPG, ADPglucose.

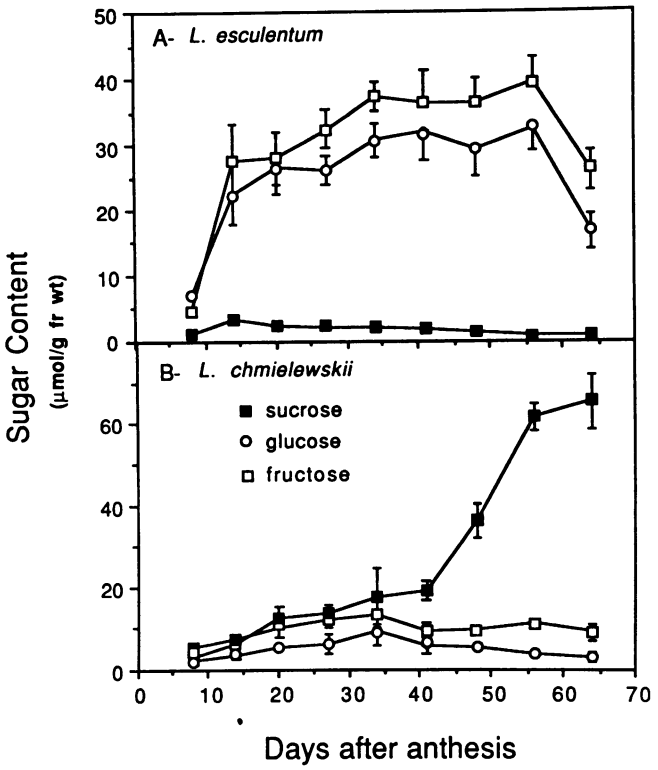

FIG. 2. Levels of soluble carbohydrate in *L. esculentum* (A) and *L. chmielewskii* (B) fruit throughout development. Each point represents the mean of three values ± SE.

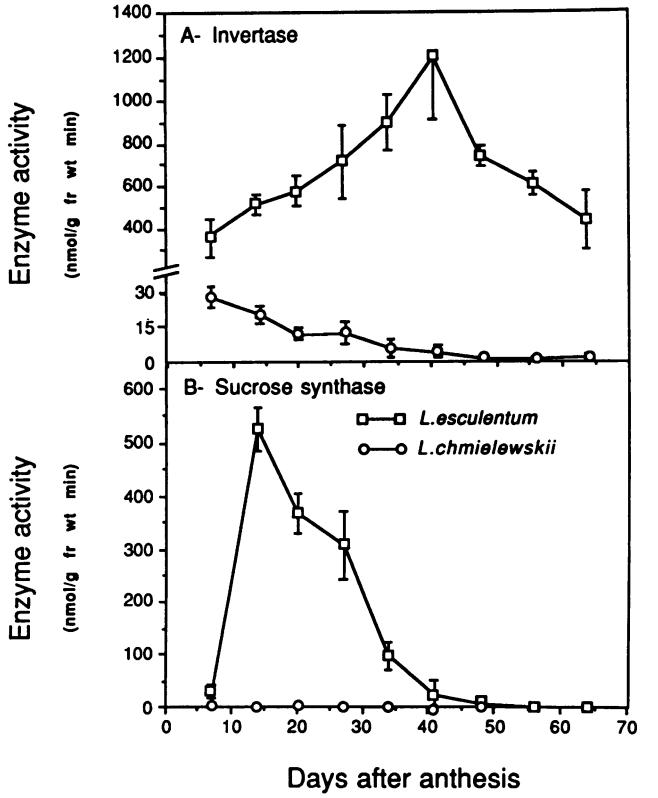

FIG. 3. Levels of invertase (A) and sucrose synthase (B) throughout development of *L. esculentum* and *L. chmielewskii* fruit. Each point represents the mean of three values ± SE.

reported (14), sucrose synthase levels were high in *L. esculentum* during early fruit development (Fig. 3B). However, in *L. chmielewskii* sucrose synthase activity was not detectable at any time during fruit development. Taken together, the low levels of invertase and sucrose synthase in *L. chmielewskii* suggest that this species may have a limited capacity to metabolize imported sucrose, thus resulting in the direct accumulation of sucrose in the fruit. The absence of sucrose synthase activity further indicates an essential role for invertase in generating hexose for maintenance of tissue metabolism and cell growth in *L. chmielewskii*.

Translocation of Asymmetric-Labeled Sucrose. To determine whether sucrose accumulated in *L. chmielewskii* fruit was derived from metabolism and resynthesis of sucrose or from the direct storage of imported sucrose, translocation and assimilation of [^{3}H]-(fructosyl)-sucrose was examined. The maintenance of the asymmetry of sucrose along the translocation path is often interpreted as an indication that sucrose has not undergone hydrolysis and resynthesis during transport (5). If hydrolysis and resynthesis of the asymmetric sucrose is occurring, the ^{3}H-label would become randomized between glucose and fructose due to hexose isomerases. The resultant sucrose would then be symmetrically labeled. In long-term labeling experiments (12 h) in *L. esculentum*, [^{3}H]-(fructosyl)-sucrose was hydrolyzed in the leaf, as evidenced by the appearance of ^{3}H-hexose (Table I). Sucrose was apparently resynthesized in the leaf resulting in [^{3}H]sucrose with a glucose/fructose (g/f) ratio of 0.42 as compared to a g/f ratio of 0.01 in the applied [^{3}H]-(fructosyl)-sucrose. The g/f ratio along the translocation pathway in *L. esculentum* increased to approximately 1.0 in the fruit. This result suggests that the small amount of sucrose found in *L. esculentum* fruit arises from hydrolysis and resynthesis of sucrose either along the translocation path or in the fruit. In *L. chmielewskii*, somewhat less [^{3}H]-(fructosyl)-sucrose was hydrolyzed and resynthesized in the leaves as evidenced by the appearance of [^{3}H]hexose and some loss of asymmetric labeling (Table I). This result differs from shorter term labeling experiments (*i.e.* 3–6 h compared to 12 h used here) where we observed maintenance of asymmetric labeling during translocation (4). The longer term labeling used here resulting in loss of asymmetry is probably due to metabolism, storage, and remobilization of carbohydrate along the translocation path (8). Even in long-term labeling experiments, sucrose along the translocation path in *L. chmielewskii* remains asymmetrically labeled (Table I), suggesting that sucrose is not hydrolyzed and resynthesized in fruit tissue but rather may be imported and stored directly in the fruit without intervening metabolism.

The labeling patterns observed with [^{3}H]-(fructosyl)-sucrose also indicated that sucrose was the translocated sugar, since most of the radioactivity in the translocation path (stem) was associated with sucrose. As expected, most of the radioactivity in the fruit of *L. chmielewskii* was in sucrose and for *L. esculentum*, glucose and fructose were the major carbohydrates labeled. The incorporation of radioactivity into starch in the fruit indicated that imported carbohydrate does contribute to starch biosynthesis in tomato fruit.

CONCLUSION

The present study has shown substantial differences in carbohydrate composition and metabolism between *L. esculentum* and *L. chmielewskii* that may contribute to sink activity of the fruit. In early fruit development, starch transiently accumulated in both species, but to a much lower level in *L. chmielewskii*. The low level of ADPG pyrophosphorylase and the high level of phosphorylase in *L. chmielewskii* probably contribute to the low level of starch accumulation.

The major difference observed between species was that *L. chmielewskii* accumulates sucrose whereas *L. esculentum* accu-

Table I. *Distribution of Radioactivity in Different Parts of Tomato Plants after Labeling a Source Leaf for 12 h with [^{3}H]-(fructosyl)-sucrose*

Fruit samples were analyzed for apoplastic (apo) and symplastic (sym) sugars as described in "Materials and Methods." Symplastic sugars were extracted in ethanol and represent cytoplasmic and vacuolar sugars.

Plant Part	Percent of Total Radioactivity				
	Starch	Sucrose	Glucose	Fructose	g/f[a] (of sucrose)
L. esculentum					
Leaves	15.5	28.8	13.4	42.4	0.42
Stems	23.9	61.9	7.0	7.2	0.75
Fruits (apo)		29.1	32.2	36.4	1.03
Fruits (sym)	11.7	14.5	32.8	35.3	0.92
L. chmielewskii					
Leaves	11.1	31.6	16.3	41.2	0.35
Stems	34.7	61.5	1.7	2.1	0.35
Fruits (apo)		91.7	3.5	4.9	0.36
Fruits (sym)	8.8	75.6	6.7	8.9	0.35

[a] [^{3}H]glucose/[^{3}H]fructose ratio after treating the sucrose HPLC fraction with invertase.

mulates hexose. This difference in soluble carbohydrate composition most likely results from the lower activities of both invertase and sucrose synthase in *L. chmielewskii*. Translocation and assimilation of asymmetrically labeled sucrose indicated that sucrose is metabolized along the translocation pathway in *L. esculentum* whereas in *L. chmielewskii* fruit sucrose may be imported and stored without intervening metabolism.

Sucrose, as opposed to hexose, accumulation may contribute to the high soluble solids content of *L. chmielewskii* fruit in several ways. Based on osmotic considerations, *L. chmielewskii* can accumulate twice as much soluble carbohydrate (when expressed on a glucose equivalent basis) as *L. esculentum* and maintain an equivalent osmotic potential. To the extent that the tomato fruit behaves as an osmometer, the *L. chmielewskii* fruit will accumulate less water, resulting in a higher soluble carbohydrate concentration in the fruit. It has also been suggested that cell turgor regulates sink activity (17). Sucrose, relative to hexose, accumulation will result in lower turgor for equivalent levels of soluble carbohydrate accumulation and so may serve to promote sink activity. Finally, especially in the presence of low levels of invertase and sucrose synthase as found in *L. chmielewskii*, sucrose is less metabolically active than hexoses and so may be inaccessible for loss through respiration. Previous studies have suggested that high invertase activity is associated with high rates of carbon import in tomato fruit (16). However, if sucrose accumulation in *L. chmielewskii* is an important factor in the accumulation of high soluble carbohydrate concentration, our results suggest that low, rather than high, invertase levels should favor increased accumulation of soluble carbohydrate.

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