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# Expression of Acid Invertase Gene Controls Sugar Composition in Tomato (*Lycopersicon*) Fruit[1]

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**A wild tomato species, *Lycopersicon chmielewskii*, accumulates high levels of soluble sugar in mature fruit and, unlike the domesticated tomato species, *Lycopersicon esculentum*, accumulates sucrose rather than glucose and fructose. Genetic and biochemical analyses of progeny resulting from a cross of *L. chmielewskii* with *L. esculentum* have previously indicated that the trait of sucrose accumulation is controlled by a single recessive gene and is associated with low levels of acid invertase protein in the developing fruit. Analysis of progeny from the $BC_2F_3$ generation from the *L. esculentum* × *L. chmielewskii* cross revealed that sucrose-accumulating fruit accumulate sugar in two phases corresponding to fruit expansion and fruit maturation and that the majority of the sucrose was stored in the latter phase after the fruit had reached maximum size. The only significant enzymic difference between the sucrose-accumulating and hexose-accumulating fruit was the lack of acid invertase activity in sucrose-accumulating fruit. Sucrose phosphate synthase activity did not increase in the sucrose-accumulating fruit during late development when the rate of sucrose accumulation increased. The lack of acid invertase activity in sucrose-accumulating fruit was correlated with inheritance of the *L. chmielewskii* acid invertase gene and the absence of acid invertase mRNA in developing fruit. This suggests that the *L. chmielewskii* invertase gene is transcriptionally silent in fruit and that this is the basis for sucrose accumulation in progeny derived from the interspecific cross of *L. esculentum* and *L. chmielewskii*.**

Carbohydrate content and composition are important determinants of tomato fruit quality both in terms of flavor, which is determined largely by the concentration of sugars and acids (Stevens et al., 1977), and as a major component of soluble solids, which contribute to tomato processing quality (Davies and Hobson, 1981). The tomato is a particularly attractive species in which to study metabolism related to soluble carbohydrate accumulation because of the natural genetic variation and well-developed genetic and physiological information in *Lycopersicon esculentum* and related species.

We have previously studied a wild tomato relative, *Lycopersicon chmielewskii*, from the subgenus *Eriopersicon*, that produces small green fruit. *L. chmielewskii* has approximately twice the soluble sugar concentration in mature fruit compared with that of the domestic tomato, *L. esculentum* (Rick, 1974; Yelle et al., 1988). In contrast to *L. esculentum*, *L. chmielewskii*, as well as *Lycopersicon peruvianum* and *Lycopersicon hirsutum* fruit, accumulates primarily Suc, rather than Glc and Fru (Davies, 1966; Yelle et al., 1988). The trait of Suc accumulation in *L. chmielewskii* and *L. hirsutum* has been suggested to be recessive and monogenic (Yelle et al., 1991; Chetelat et al., 1993; J.R. Stommel, personnal communication) and is associated with low levels of both acid invertase activity and immunologically detectable protein (Yelle et al., 1988). The predominant form of acid invertase in ripening tomato fruit, a 52-kD protein, was localized to the vacuole in protoplast experiments (Konno et al., 1993); however, intercellular washing of pericarp discs suggested that it was localized in the cell wall (Sato et al., 1993).

Storage of Suc may contribute to the ability of tomatoes to store higher amounts of soluble sugars. Indeed, many storage organs that accumulate high levels of sugar accumulate primarily Suc (Hatch and Glasziou, 1963; Giaquinta, 1979; Schaffer et al., 1987; Stommel and Simon, 1989). Suc storage may contribute to high sugar levels because it has half the osmolarity as the equivalent Glc and Fru (Steingröver, 1983) and is less metabolically accessible than hexose sugars to respiratory loss (Salerno and Pontis, 1978). However, there is a hexose-accumulating tomato species, *Lycopersicon pimpinellifollium*, that also has a very high sugar content compared with the cultivated species (Manning and Maw, 1975). Furthermore, Suc levels have been negatively correlated with the rate of carbon import in tomato fruit (Walker and Thornley, 1977; Walker et al., 1978). These observations have led to speculations that Suc hydrolysis primarily by acid invertase may determine the rate and extent of Suc storage in tomato fruit (Walker et al., 1978).

In contrast, low levels of acid invertase are associated with high levels of Suc accumulation in *L. hirsutum* (Miron and Schaffer, 1991), *L. peruvianum* (Stommel, 1992), and *L. chmielewskii* (Yelle et al., 1988). In addition, an increase in Suc-P synthase is associated with accelerated rates of Suc storage late in fruit development of *L. hirsutum* (Miron and Schaffer, 1991). The association of Suc-P synthase activity and Suc storage has lead to the proposal that Suc storage may occur by Suc hydrolysis and resynthesis, with Suc-P synthase activity increasing the rate of Suc accumulation by maintaining the hexose gradient between the apoplast and cytosol (Miron and Schaffer, 1991).

In the present study we sought to characterize the biochemical and molecular aspects of the trait of Suc accumulation found in *L. chmielewskii*. To minimize nonrelated phenotypic characteristics, plants were used in which the trait of Suc

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Abbreviation: RFLP, restriction fragment length polymorphism.

accumulation from *L. chmielewskii* was introduced into *L. esculentum*. The results of this study indicate that Suc-accumulating fruit accumulated sugar in two phases corresponding to fruit expansion and fruit maturation and that the majority of the Suc was stored during the latter phase, after the fruit had reached maximum size. Suc-P synthase activity was not correlated with Suc accumulation even in late developmental stages, but reduced acid invertase activity was associated with Suc accumulation. Furthermore, the absence of acid invertase activity in Suc-accumulating fruit results from the introgression of the *L. chmielewskii* invertase gene and failure of invertase mRNA to accumulate in fruit.

## MATERIALS AND METHODS

### Plant Material

$BC_1F_2$ progeny of a cross between *Lycopersicon esculentum* (cv UC82B) and *Lycopersicon chmielewskii* (LA1028) (Yelle et al., 1991) were backcrossed to *L. esculentum* (cv UC204C) and then selfed to produce the $BC_2F_2$ generation. Individual plants in the $BC_2F_2$ generation were selected by RFLP analysis that were homozygous for either the allele for the trait of Suc accumulation (*sucr/sucr*) or the allele for the trait of hexose accumulation ($sucr^+/sucr^+$) (Chetelat et al., 1993). These plants were self-pollinated, and the resulting progeny formed the $BC_2F_3$ families used here: GH5124, GH5129, GH5138, and GH5143.

The plants were grown in a screenhouse during summer months and in a greenhouse with supplemental light during fall and winter months. Fruit were tagged at anthesis and harvested at 1-week intervals from 3 to 9 weeks. Upon harvest, fruit diameter and weight were recorded, and the pericarp was frozen in liquid nitrogen, or in the case of *L. chmielewskii*, the entire fruit was frozen. Tissue samples were stored at −80°C before analysis.

### Carbohydrate Measurements

Carbohydrate was extracted from 90 to 125 mg of tomato pericarp in a microfuge tube with 0.5 mL of 80% ethanol and boiled for 20 min. Following centrifugation (6 krpm, 5 min, microfuge), the ethanol supernatant was transferred to a fresh tube. The residue was reextracted with 0.5 mL of boiling 80% ethanol, and the ethanol supernatants were pooled. The twice-extracted tissue pellet was stored at −20°C for starch determination.

For soluble sugar analysis, the 80% ethanol extract was evaporated under vacuum to dryness (SpeedVac; Savant Instruments), and the residue was dissolved in 200 μL of $H_2O$. This aqueous fraction was extracted twice with chloroform:isoamyl alcohol (24:1) and filtered through a 0.2-μm or 0.4-μm microfuge spin filter (Gelman, Ann Arbor, MI, or PGC Scientific, Gaithersburg, MD) before HPLC analysis. Sugars were identified and quantified by chromatography on an Aminex carbohydrate column (300 × 7.8 mm) and detected with a refractive index detector (Altex 156). Concentrations were calculated from peak heights using Glc, Fru, and Suc standards.

Starch was determined in the ethanol-insoluble fraction by initially drying and resuspending the pellet in 200 μL of 0.2 N KOH and boiling for 30 min. The extract was brought to approximately pH 5.5 with 50 μL of 1 M acetic acid, and then 0.182 mg of amyloglucosidase lyophilizate from *Aspergillus niger* (70% protein; Boehringer-Mannheim No. 208 469) and 1.62 μg of α-amylase lyophilizate from *Bacillus amyloliquefaciens* (75% protein; Boehringer-Mannheim No. 161 764) were added for a total volume of 300 μL. Reactions were incubated at 37°C overnight, after which the insoluble material was removed by centrifugation (5 min, 14 krpm, microcentrifuge). Nelson's reducing sugar assay was used as described below to assay 100 μL of 1:10 or 1:100 diluted samples.

### Enzyme Assays

Three pericarp samples from separate fruit were extracted for each data point. Enzymes were extracted from 0.95 to 1.2 g of minced frozen pericarp by homogenizing for 1 min on ice in 200 mM Hepes/NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 0.5 mg $mL^{-1}$ of BSA, 0.005% Triton X-100, 1 M NaCl, 1% insoluble PVP, and 5 mM DTT using a tissumizer (Tekmar, Cincinnati, OH) with an S25–8G probe on 70% power. Homogenates were quickly desalted at 4°C by centrifuging 1 mL through a 5-mL Sephadex G-50 spin column (Helmerhorst and Stokes, 1980) equilibrated in 50 mM Hepes/NaOH (pH 7.5), 5 mM $MgCl_2$, 0.5 mg $mL^{-1}$ of BSA, and 5 mM DTT. Desalted extracts were assayed immediately.

Suc synthase and Suc-P synthase assays were performed in a manner similar to that previously described (Hubbard et al., 1989; Miron and Schaffer, 1991). Suc synthase and Suc-P synthase reactions and controls contained 40 μL of desalted extract in a total volume of 70 μL. Suc synthase assays contained 50 mM Hepes/NaOH (pH 7.5), 15 mM $MgCl_2$, 25 mM Fru, and 25 mM UDP-Glc. Suc-P synthase assays contained 50 mM Hepes/NaOH (pH 7.5), 15 mM $MgCl_2$, 25 mM Fru-6-P, 25 mM Glc-6-P, and 25 mM UDP-Glc. Controls were boiled for 10 min immediately after the addition of the desalted enzyme extract. The reactions were incubated for 30 min at 37°C and then stopped by transfer to a boiling water bath. Reactions and controls were stored at −20°C until the Suc was assayed using the anthrone assay (Van Handel, 1968). To the 70-μL sample, 70 μL of 30% KOH was added, boiled for 10 min, and cooled. Anthrone reagent (1 mL; 76 mL of sulfuric acid, 30 mL of $H_2O$, and 150 mg of anthrone) was then added, and the tubes incubated for 20 min at 40°C. Absorbance was measured with a microplate reader (Bio-Rad model 3550-UV) at 650 or 595 nm, with dilutions made with 70% sulfuric acid as necessary, and compared to Suc standards.

Acid invertase assays contained either 20 or 5 μL of desalted extract in a total volume of 100 μL. The final assays contained 500 mM sodium acetate/NaOH (pH 4.5) and 120 mM Suc. Both reactions and controls were neutralized with the addition of 30 μL of 2.5 M Tris base before boiling to prevent acid hydrolysis of the Suc. Controls were boiled immediately, and the reactions were incubated at 37°C for 30 min before boiling. Reaction and controls were stored at −20°C until the reducing sugar assay was performed.

Reducing sugars in the acid invertase assays were measured using the Somogyi modification of Nelson's reducing sugar assay (Nelson, 1944; Somogyi, 1952). Copper-tartrate reagent

(1 mL) was added to the 130-μL sample, boiled for 10 min, and cooled. The sample mix was then added to 1 mL of the arsenomolybdate reagent in a 13- × 100-mm tube and vortexed. Absorbance was read with a microplate reader at 595 or 570 nm and compared to Glc standards.

### RNA Hybridization Analysis

RNA was extracted from fruit pericarp by homogenizing 10 g of pulverized frozen tissue from several fruit in homogenization buffer containing 10 mL of 1 M Tris/HCl (pH 9.0) saturated phenol with 0.1% 8-hydroxyquinoline, 10 mL of chloroform:isoamyl alcohol (24:1), and 20 mL of 1 M Tris/HCl (pH 9.0) with 5% β-mercaptoethanol. Tissue was homogenized for 2 min on ice with a Tekmar tissumizer with a 10N probe at 100% power. After the homogenate was centrifuged (10 min, 900*g*, clinical centrifuge), the aqueous phase was removed to a new tube, and the phenol/chloroform phase was reextracted with 20 mL of 1 M Tris/HCl (pH 9.0) with 5% β-mercaptoethanol. RNA was precipitated overnight from the pooled aqueous phases with 0.1 volume of 3 M sodium acetate and 2.5 total volumes of cold ethanol. RNA was then pelleted (12,000*g*, 20 min), vacuum dried, and dissolved in 300 μL of 100 mM Tris/HCl (pH 8.0). The RNA was extracted with phenol equilibrated in 100 mM Tris/HCl (pH 8.0) and chloroform:isoamyl alcohol (24:1) in a ratio of 1:1 and with chloroform:isoamyl alcohol alone. Extracted RNA was brought to 2 M $LiCl_2$, stored overnight at −20°C and pelleted (28,000*g*, 20 min), and the pellet was washed in 70% ethanol. After the pellet was dried, polysaccharides were removed by dissolving the pellet in 300 μL of $H_2O$, adding 150 μL ethanol, and centrifuging (10 min, 14 krpm, microfuge). The supernatant was transferred to a fresh tube, and 120 μL of 5 M ammonium acetate (pH 5.5) and 900 μL of ethanol was added. The RNA was centrifuged out of the solution (10 min, 14 krpm, microfuge), redissolved in $H_2O$, and stored in 70% ethanol at −80°C.

RNA concentration was determined by $A_{260}$. Slot blots were performed with a MinifoldII slot blot system (Schleicher & Schuell) with 10 μg of total RNA per slot following procedures detailed in the apparatus manual. The control slot contained 10 μg of Brome mosaic virus RNA (BRL). Formaldehyde-agarose RNA gel electrophoresis (Nevins and Wilson, 1981) was performed with 10 μg of total RNA per lane. RNA was transferred to Hybond-N membrane (Amersham) in both cases and prehybridized at 42°C for at least 18 h in 50% formamide, 5× Denhardt's solution, 5× SSPE, 0.5% SDS, and 0.2 mg $mL^{-1}$ of base-denatured salmon sperm DNA (Sargent, 1987). Hybridizations were done in an identical solution at 42°C for at least 24 h. Blots were washed at 42°C with 0.1% SDS, 5× SSC twice for 15 min, at 42°C with 1× SSC, 0.1% SDS once for 30 min, and finally at 65°C with 0.5× SSC, 0.1% SDS for 10 min. Blots were exposed to Kodak X-Omat film at −80°C with an intensifying screen.

### DNA Hybridization Analysis

Genomic DNA was isolated from the leaves of one individual from each family of tomato plants (Murray and Thompson, 1980). Standard protocols were used for the DNA digestion and Tris-borate-EDTA DNA gel electrophoresis (Sambrook et al., 1989). DNA was transferred to Hybond-N membrane and hybridized according to the Hybond-N protocol. The blot was washed at a final stringency of 0.1× SSC at 65°C, also according to the Hybond-N protocol.

### Probes

Invertase DNA probes were produced by random priming (Boehringer-Mannheim or BRL) of isolated tomato acid invertase cDNA with [$\alpha$-$^{32}$P]dCTP and [$\alpha$-$^{32}$P]dATP (New England Nuclear). The sequence of tomato acid invertase, TIV1, has been previously published (Klann et al., 1992).

## RESULTS

### Sugar Accumulation

The trait of Suc accumulation in *L. chmielewskii* fruit is controlled by a single recessive gene, and its chromosomal location has been determined by RFLP mapping to be on chromosome 3 linked to the RFLP marker TG102 (Chetelat et al., 1993). The *L. esculentum* allele is designated *sucr*$^+$, and the *L. chmielewskii* allele is designated *sucr*. A series of crosses were made between *L. esculentum* and *L. chmielewskii* to transfer the trait of Suc accumulation (*sucr*) from *L. chmielewskii* into a background in which 87.5% of the genome was contributed by *L. esculentum*. In the $BC_2F_2$ generation, plants were selected that were homozygous for either the allele for Suc accumulation (*sucr/sucr*) or the allele for hexose accumulation (*sucr*$^+$/*sucr*$^+$) and self-pollinated to produce $BC_2F_3$ families of plants. Although *L. esculentum* and *L. chmielewskii* differ in many phenotypic characteristics, the $BC_2F_3$ families are more phenotypically similar. For example, whereas fruit size of the parents *L. esculentum* and *L. chmielewskii* differed widely, fruit size was similar among all $BC_2F_3$ families at all stages of development (Fig. 1). All fruit exhibited a period of fruit expansion, followed by a period of fruit maturation and ripening.

To determine the developmental changes in carbohydrate accumulation in the $BC_2F_3$ families and the parental lines, carbohydrate composition of fruit of two *sucr/sucr* families, of two *sucr*$^+$/*sucr*$^+$ families, and of *L. esculentum* and *L. chmielewskii* was assessed during fruit development. *L. esculentum* fruit had a constant Glc and Fru concentration on a fresh weight basis throughout the period of fruit development assayed, suggesting that sugar accumulation paralleled water uptake (Fig. 2). The Suc concentration in *L. esculentum* fruit remained constant and low through fruit development. In *L. chmielewskii* fruit, however, two phases of sugar accumulation corresponding to the periods of fruit expansion and fruit maturation were evident. Suc concentration increased at a continuous but low rate during early development (3–6 weeks) and at a higher rate during the final weeks of development (7–9 weeks) (Fig. 2). The concentration of hexose sugars remained moderate and constant throughout *L. chmielewskii* development. The general pattern of sugar accumulation in *L. chmielewskii* fruit observed here is similar to that previously observed in this species (Yelle et al., 1988) and is similar to fruit of two other wild tomato species that accumulate Suc, *L. peruvianum* and *L. hirsutum* (Miron and Schaf-

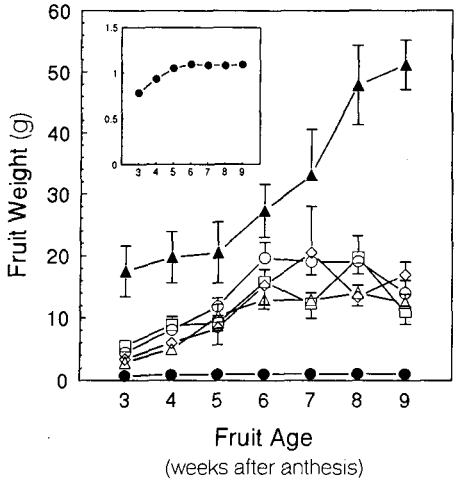


**Figure 1.** Change in fruit weight during development of *L. esculentum* (▲), *L. chmielewskii* (●), and the $BC_2F_3$ families GH5124 (◇), GH5129 (□), GH5138 (△), and GH5143 (○). Inset contains data from *L. chmielewskii*. Each data point is the mean ± SE of all harvested fruit.

fer, 1991; Stommel, 1992). Both of these species had little increase in Suc concentration during the first half to two-thirds of development, followed by a sharp increase in Suc concentration in late development, suggesting that fruit of these wild species may also exhibit two phases of sugar accumulation.

Fruit of the $BC_2F_3$ families of plants showed sugar accumulation patterns that were similar to the parental lines. The *sucr/sucr* fruit accumulated predominantly Suc with moderate levels of Glc and Fru, in a two-stage manner very similar to *L. chmielewskii* fruit. The $sucr^+/sucr^+$ plants accumulated predominantly Glc and Fru with very low levels of Suc throughout development, in a manner similar to *L. esculentum* fruit. The similarity in the pattern of sugar accumulation of the $BC_2F_3$ *sucr/sucr* families and *L. chmielewskii* suggests that the trait of Suc accumulation had been transferred largely intact into the *L. esculentum* background.

Starch concentration during fruit development was also determined (Fig. 2). Although the Suc-accumulating $BC_2F_3$ families did accumulate somewhat more starch than the hexose-accumulating families, the highest starch-accumulating family (GH5124) did not accumulate the highest amounts of sugars later in development. It is interesting that both the parental *L. esculentum* and *L. chmielewskii* had noticeably less starch than any of the backcross families. We were unable to determine the source of this high starch phenotype, but some increase in the concentration of starch expressed on a fresh weight basis from *L. esculentum* to the $BC_2F_3$ families may be due to the decreased water uptake of these fruit.

Suc accumulation did not necessarily lead to higher sugar accumulation. Whereas the Suc-accumulating family (GH5138) accumulated the highest level of soluble carbohydrate (98.0 mg $g^{-1}$ fresh weight) by 8 weeks after anthesis, the other Suc-accumulating family (GH5124) (44.3 mg $g^{-1}$ fresh weight) was very similar to the hexose-accumulating families GH5129 (36.6 mg $g^{-1}$ fresh weight) and GH5143 (52.5 mg $g^{-1}$ fresh weight) at this time. It is known that in *L. chmielewskii* high soluble solids is a polygenic trait and presumably involves the action of multiple metabolic determinants (Paterson et al., 1988). Factors other than Suc accumulation that contribute to higher total sugars may also be segregating in this population and could obscure the effect of Suc accumulation.

### Enzyme Activity

To further assess the biochemical basis of the trait of Suc accumulation, pericarp extracts of developing fruit were assayed for acid invertase, Suc synthase, and Suc-P synthase. Acid invertase was the only enzyme that differed significantly between the $sucr^+/sucr^+$ and *sucr/sucr* plants (Fig. 3). Suc-accumulating families showed the lowest levels of acid invertase activity throughout fruit development, whereas hexose-accumulating families had moderate amounts of acid invertase activity during the first two-thirds of fruit development, with increases up to 10-fold during fruit ripening.

Suc synthase activity paralleled starch accumulation during fruit development in the backcross families and the parents.

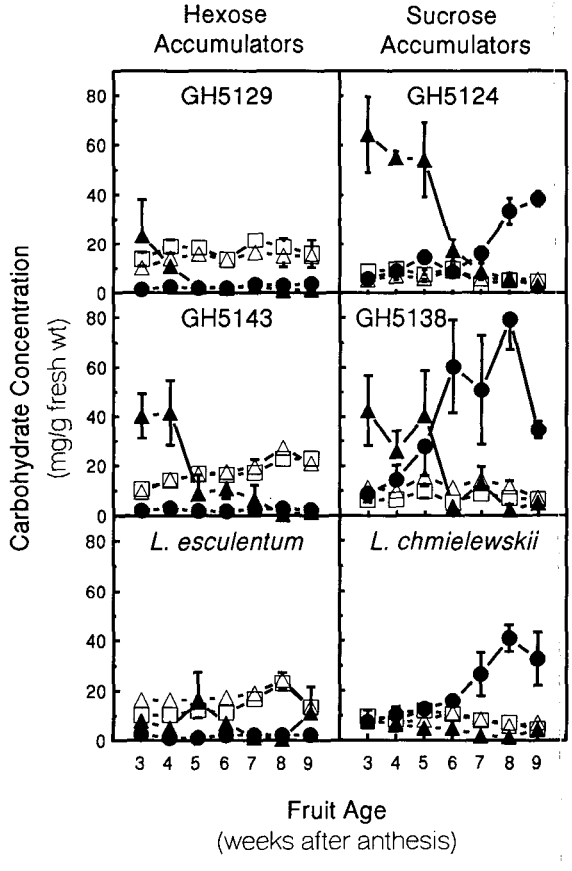


**Figure 2.** Analysis of carbohydrate composition in the fruit pericarp of *L. esculentum*, *L. chmielewskii*, two hexose-accumulating families (GH5129 and GH5143), and two Suc-accumulating families (GH5124 and GH5138). Suc (●), Glc (△), Fru (□), and starch (▲) were determined during fruit development. Each data point is the mean ± SE of three pericarp samples, each from a different fruit.

Starch concentration began to decrease approximately 1 week after Suc synthase activity decreased, which agrees with previous reports correlating Suc synthase activity with starch synthesis (Robinson et al., 1988; Sun et al., 1992). The higher Suc synthase activity in the *sucr/sucr* fruit generally corresponds to the higher starch concentrations in those fruit (Fig. 2).

The pattern and level of Suc-P synthase activity did not differ between the *sucr*$^+$/*sucr*$^+$ and *sucr/sucr* plants. In general, the activity of Suc-P synthase in the backcross families was higher during the first half of fruit development and decreased by 40 to 95% during fruit maturation and ripening, in a manner similar to *L. esculentum* fruit. In contrast, *L. chmielewskii* had low but constant levels of Suc-P synthase activity throughout fruit development. Similar levels of Suc-P synthase activity have been previously measured in *L. esculentum* fruit (Miron and Schaffer, 1991; Stommel, 1992). However, the previously reported increase in Suc-P synthase activity during fruit maturation (Miron and Schaffer, 1991; Dali et al., 1992) was not observed in this study, even though the $BC_2F_3$ population used here was derived from the same backcross stocks studied by Dali et al. (1992).

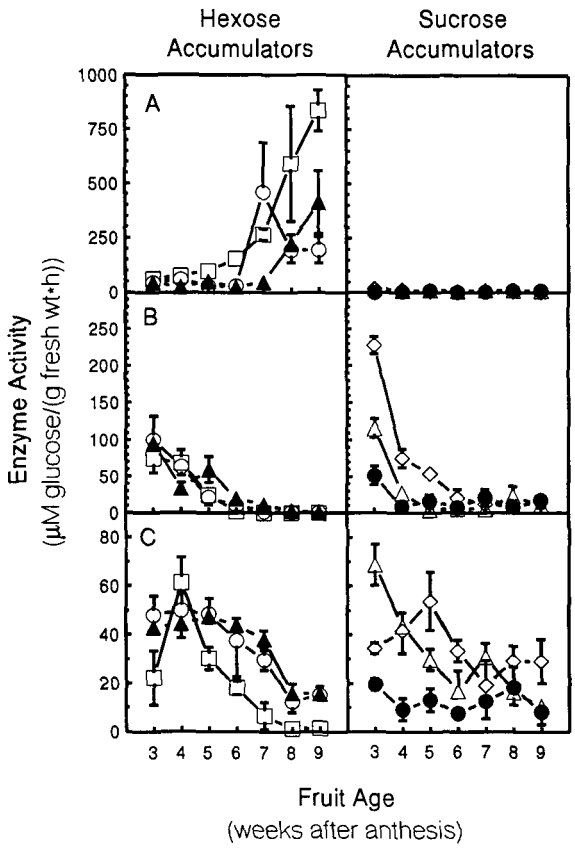


**Figure 3.** Activity of acid invertase (A), Suc synthase (B), and Suc-P synthase (C) in fruit pericarp. Proteins from *L. esculentum* (▲), *L. chmielewskii* (●), GH5124 (◇), GH5129 (□), GH5138 (△), and GH5143 (○). Each data point is the mean ± SE of three pericarp samples, each from a different fruit.

### RNA Accumulation

To better understand the basis of invertase deficiency in *sucr/sucr* fruit, the level of invertase mRNA was determined in developing fruit. The cDNA that encodes the predominant form of acid invertase in ripening fruit has been isolated (Klann et al., 1992) and was used to probe northern blots of total RNA. The cDNA encoding acid invertase hybridized to a single RNA species of 2.3 kb in *L. esculentum* (Fig. 4A). Acid invertase mRNA accumulation in *L. esculentum* was moderate through the early stages of fruit development (3–6 weeks) and increased dramatically upon fruit ripening (6–9 weeks). The size and expression patterns of the acid invertase mRNA are similar to what has been observed before in *L. esculentum* fruit (Endo et al., 1990; Elliot et al., 1993). The level of acid invertase mRNA in young *L. esculentum* fruit was similar to the levels found in roots, green shoots, and etiolated shoots of *L. esculentum* (not shown). In contrast, invertase mRNA accumulation was undetected in *L. chmielewskii* at all stages of fruit development assayed (Fig. 4A).

The backcross families showed the same general patterns of acid invertase mRNA accumulation as the parents during fruit development (Fig. 4B). The lack of invertase mRNA in the Suc-accumulating fruit was presumably responsible for the reduction in the acid invertase activity in these fruit. The levels of invertase mRNA closely paralleled the levels of acid invertase enzyme activity within fruit of the $BC_2F_3$ families (Fig. 3).

### Genomic DNA Analysis

Genomic Southern blots were probed with the acid invertase cDNA to determine whether the *L. chmielewskii* genome contained an invertase gene corresponding to that in *L. esculentum*. The acid invertase cDNA hybridized to restriction fragments in both *L. esculentum* and *L. chmielewskii* genomic DNA with equal intensity (Fig. 5). At high stringency (Tm −5°C), only one genomic restriction fragment in each species hybridized to the invertase cDNA, suggesting the presence of a single closely related gene in each genome. At low stringency, a number of weakly hybridizing restriction fragments were evident (not shown).

The *Eco*RI digest of genomic DNA revealed an RFLP that was used to distinguish the *L. esculentum* and *L. chmielewskii* acid invertase alleles in genomes of the backcross families. The two Suc-accumulating families (*sucr/sucr*) were both homozygous for the *L. chmielewskii* allele of the invertase gene, whereas the hexose-accumulating families (*sucr*$^+$/*sucr*$^+$) were homozygous for the *L. esculentum* allele. These results indicate that the *L. chmielewskii* allele of the invertase gene cosegregates with the trait of Suc accumulation in the $BC_2F_3$ families.

## DISCUSSION

A major distinction between the pattern of sugar accumulation in Suc- and hexose-storing tomato fruit is the timing. Hexose-accumulating fruit cease to accumulate appreciable amounts of sugar after fruit have reached mature size, whereas Suc accumulators continue to accumulate sugar late in development. Both *L. chmielewskii* and the Suc-accumu-

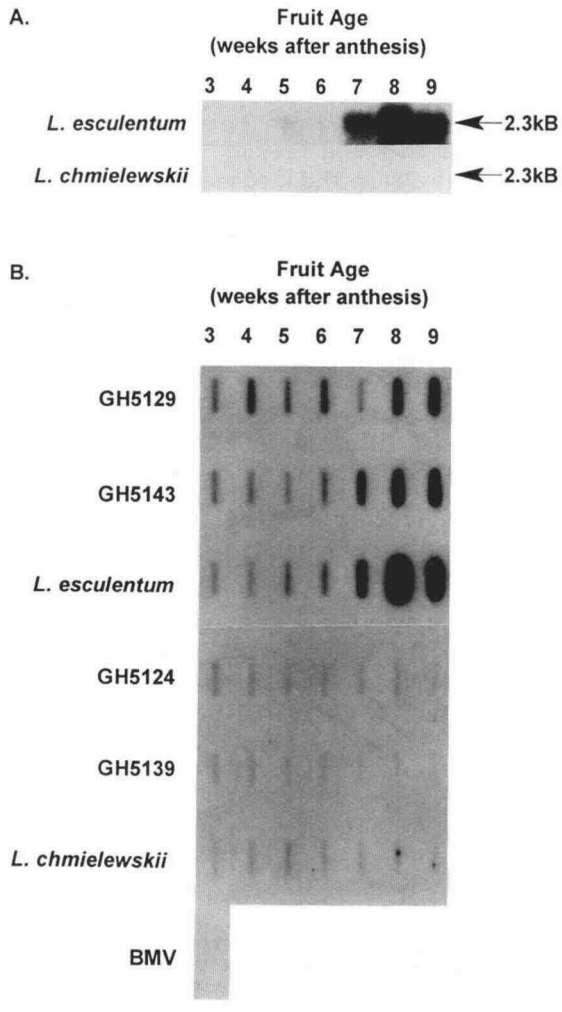


**Figure 4.** Northern blot analysis of total RNA from pericarp during fruit development. Formaldehyde-agarose RNA gel electrophoresis analysis of *L. esculentum* and *L. chmielewskii* fruit RNA, 10 μg total RNA per lane (A), and slot blot analysis of fruit RNA from *L. esculentum*, *L. chmielewskii*, and the $BC_2F_3$ families, 10 μg total RNA per slot (B). Blots were hybridized with the cDNA for tomato acid invertase (TIV1) and washed with 0.5× SSC at 65°C. BMV, Brome mosaic virus.

lating $BC_2F_3$ fruit, therefore, have two stages of accumulation: the first during fruit expansion and the second during fruit maturation.

Other aspects of tomato fruit development also occur in two stages. The metabolic pathway of Suc storage in Suc-accumulating fruit changes at approximately the same time as when the second stage of Suc accumulation begins. When Suc-accumulating tomatoes derived from a cross between *L. esculentum* and *L. chmielewskii* were fed asymmetrically labeled Suc, Suc was found to be stored directly until about 6 weeks after anthesis, after which time Suc was broken down and resynthesized before storage (Dali et al., 1992).

The cellular pathway of sugar movement may also change midway through tomato fruit development. At 2 weeks after anthesis, transfer of Suc from the vascular bundle is suggested to be symplastic, based on the calculated facilitated membrane transfer of the combined vascular membrane surface area, the distribution of plasmodesmata, and the observed rates of Suc transfer (Offler and Horder, 1992). At about 4 weeks after anthesis, vascular structure had changed such that similar calculations indicated that the observed rates of Suc transfer were possible, based solely on unloading to the apoplast (Offler and Horder, 1992). The change in vascular architecture that occurs midway through fruit development may, therefore, contribute to a redirection of Suc movement from a symplastic to an apoplastic path.

The timing of these transitions in sugar accumulation suggests a model of sugar uptake in developing tomato fruit. In this model, the early stage of fruit development is characterized by net water accumulation in fruit, largely symplastic transport of Suc and its storage without intervening metabolism. The presence of Suc synthase activity in the cytosol during early development suggests that some Suc must be broken down, but it is possible that the resulting hexoses are used primarily for starch metabolism and respiration, with little resynthesized to Suc. A gradient in Suc concentration between the phloem cells and the storage parenchyma may be maintained by dilution from incoming water, starch synthesis, respiration, and active Suc storage in the vacuole. In the later developmental stage, net water uptake by the fruit ceases, the movement of Suc becomes increasingly apoplastic, and the storage of resynthesized Suc begins. Suc may be hydrolyzed by apoplastic acid invertase, resynthesized by

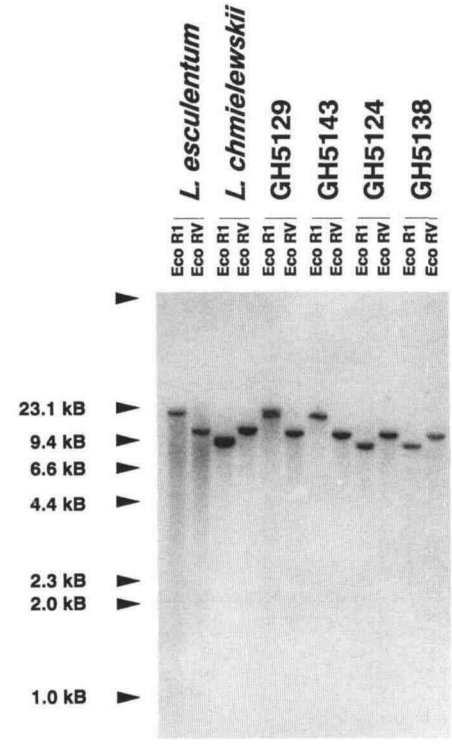


**Figure 5.** Southern blot analysis of genomic DNA from *L. esculentum*, *L. chmielewskii*, and the $BC_2F_3$ families, 10 μg DNA per lane. Blot was hybridized with the cDNA for tomato acid invertase and washed with 0.1× SSC at 65°C.

cytoplasmic Suc-P synthase, and stored in the vacuole (Miron and Schaffer, 1991). The hydrolysis and resynthesis of Suc in this latter developmental stage would lower the apoplastic concentration of Suc, thereby increasing the Suc gradient from the phloem as well as establishing a hexose gradient between the apoplast and cytoplasm (Miron and Schaffer, 1991).

Suc-P synthase activity has been suggested to determine the rate of Suc synthesis and storage in late tomato fruit development (Miron and Schaffer, 1991; Dali et al., 1992). Suc-P synthase is a determinant in the level of Suc accumulation in muskmelons (Lingle and Dunlap, 1987; Hubbard et al., 1989), banana (Hubbard et al., 1990), Asian pear (Moriguchi et al., 1992), peach, strawberry, kiwi, and mango (Hubbard et al., 1991). It does not appear, however, that the level of Suc-P synthase activity found here is a limiting factor for Suc accumulation. We observed a lower level of Suc-P synthase activity in *L. chmielewskii* than in *L. esculentum* and all of the backcross families. Furthermore, levels of Suc-P synthase activity observed in fruit from another Suc-accumulating family derived from a cross between *L. esculentum* and *L. chmielewskii* were only 5% of those observed in this study (Dali et al., 1992). Therefore, if Suc resynthesis contributes to Suc storage, levels of Suc-P synthase present in hexose-accumulating fruit are likely to be sufficient to carry out this step, even in Suc accumulators.

Suc synthase has also been suggested to be involved in Suc accumulation in storage organs. An increase in Suc synthase activity accompanies Suc storage in sugar beet (Giaquinta, 1977; Fieuw and Willenbrink, 1987), melon (Lingle and Dunlap, 1987; Schaffer et al., 1987; McCollum et al., 1988), Asian pear (Moriguchi et al., 1992), peach, and strawberry (Hubbard et al., 1991). In *L. esculentum* fruit, however, Suc synthase is generally associated with starch synthesis (Robinson et al., 1988; Wang et al., 1993). We have not observed an association between Suc synthase and Suc accumulation in the fruit of *L. chmielewskii* or plants derived from crosses with it (Dali et al., 1992).

It is certain from the present study that acid invertase plays a predominant role in Suc accumulation in tomato fruit. Not only is the enzyme activity level deficient in Suc-accumulating tomatoes, the levels of mRNA encoding acid invertase are undetectable. In contrast, invertase mRNA levels and enzyme activity are high in hexose-accumulating fruit. The low level of acid invertase mRNA in fruit is associated with inheritance of the *L. chmielewskii* invertase gene in plants segregating for Suc accumulation. Together, these results suggest that the *L. chmielewskii* invertase gene is transcriptionally silent in fruit.

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