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Antisense Inhibition of Tomato Fruit Sucrose Synthase Decreases Fruit Setting and the Sucrose Unloading Capacity of Young Fruit

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The role of sucrose synthase (SuSy) in tomato fruit was studied in transgenic tomato (*Lycopersicon esculentum*) plants expressing an antisense fragment of fruit-specific SuSy RNA (*TOMSSF*) under the control of the cauliflower mosaic virus 35S promoter. Constitutive expression of the antisense RNA markedly inhibited SuSy activity in flowers and fruit pericarp tissues. However, inhibition was only slight in the endosperm and was undetectable in the embryo, shoot, petiole, and leaf tissues. The activity of sucrose phosphate synthase decreased in parallel with that of SuSy, but acid invertase activity did not increase in response to the reduced SuSy activity. The only effect on the carbohydrate content of young fruit was a slight reduction in starch accumulation. The in vitro sucrose import capacity of fruits was not reduced by SuSy inhibition at 23 days after anthesis, and the rate of starch synthesized from the imported sucrose was not lessened even when SuSy activity was decreased by 98%. However, the sucrose unloading capacity of 7-day-old fruit was substantially decreased in lines with low SuSy activity. In addition, the SuSy antisense fruit from the first week of flowering had a slower growth rate. A reduced fruit set, leading to markedly less fruit per plant at maturity, was observed for the plants with the least SuSy activity. These results suggest that SuSy participates in the control of sucrose import capacity of young tomato fruit, which is a determinant for fruit set and development.

INTRODUCTION

Sucrose synthase (SuSy; EC 2.4.1.13) and acid invertase (AI; EC 3.2.1.26) catalyze the cleavage of sucrose in rapidly growing tomato fruit. During the past decade, evidence has increasingly indicated that SuSy is responsible for the cleavage of newly imported sucrose, controlling the import capacity of the fruit (N'tchobo et al., 1999) and the rate of starch synthesis (Wang et al., 1993a). The proposal for a central role of SuSy in rapidly growing tomato fruit is based on the profile of activity of the enzyme in the course of fruit development. Early in fruit development, SuSy activity increases, reaches a maximum at 20 to 25 days after anthesis (DAA), and then decreases. In the fruit, starch (Wang et al., 1993a), dry weight (Demnitz-King, 1993), fresh weight accumulation (Stommel, 1992; Sun et al., 1992), and sucrose import capacity (N'tchobo et al., 1999) also parallel this developmental profile. Furthermore, the correlative evidence of a regulatory role for SuSy is supported by the cytosolic localization of the enzyme (Avigad, 1982; Hawker, 1985). In the early stages of fruit development, sucrose is unloaded symplastically, directly into the cytosol of the sink cell (Damon et al., 1988; Dali et al., 1992; Ruan and Patrick, 1995). In addition, SuSy, with its UDP-transferase activity, possesses an energetic advantage over AI by forming UDP-glucose, which does not need ATP-dependent phosphorylation. In comparison, the hydrolytic activity of AI gives rise to glucose and fructose, both of which require ATP-dependent phosphorylation.

To assess the specific role of SuSy in sucrose unloading in growing tomato fruit, Wang et al. (1993a) performed inhibition experiments involving heat shock treatment of tomato plants. This treatment substantially decreased SuSy activity in fruit without affecting AI activity. Concomitant with SuSy inhibition, the fruits from heat shock–treated plants showed a decreased capacity for breakdown and incorporation of ^{14}C-sucrose into the starch fraction (Wang et al., 1993a). On the basis of these observations, the authors concluded that SuSy activity controlled the import capacity of the fruit and favored the partitioning of carbohydrates toward the starch fraction. In contrast, ^{3}H-sucrose–uptake experiments demonstrated that in tomato fruit at 10, 20, and 40 DAA, 20 to 25% of the imported sucrose is directed to the starch fraction, independently of SuSy activity (N'tchobo et al., 1999).

Mutants with reduced SuSy activity have been produced and studied in maize (Chourey and Nelson, 1976) and pea (Craig et al., 1996) as well as in transgenic potatoes (Zrenner

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et al., 1995) and transgenic *Lotus japonicus* (Skot et al., 1997) that express an antisense gene for SuSy. Unfortunately, no mutant tomato line lacking SuSy activity is currently available for study of the metabolic responses to a loss of SuSy in tomato fruit. In maize endosperm and potato tubers, inhibition of SuSy activity to <5% of that in control plants led to decreases in starch content of 40 and 70%, respectively (Chourey and Nelson, 1976; Zrenner et al., 1995). In the SuSy antisense potato plants, the lower starch accumulation was accompanied by a 50% reduction in dry matter content compared with that of the control plants (Zrenner et al., 1995). In contrast to maize endosperm and potato tubers, tomato fruit contains very high invertase activity, which is possibly responsible for hexose accumulation in the fruit (Yelle et al., 1988, 1991; Klann et al., 1993; Ohyama et al., 1995); however, invertase activity was not associated with the sink strength of the tissue (Klann et al., 1996).

In this study, we characterize carbon metabolism, fruiting capacity, and fruit growth of transgenic tomato plants expressing an antisense fragment of the fruit-specific SuSy RNA (*TOMSSF*). A biochemical analysis of the transgenic tomato plants is presented as well as physiological data on fruit productivity. We provide direct evidence that the control of SuSy activity over fruit growth rate and fruit set depends on the position of the fruits on the plant.

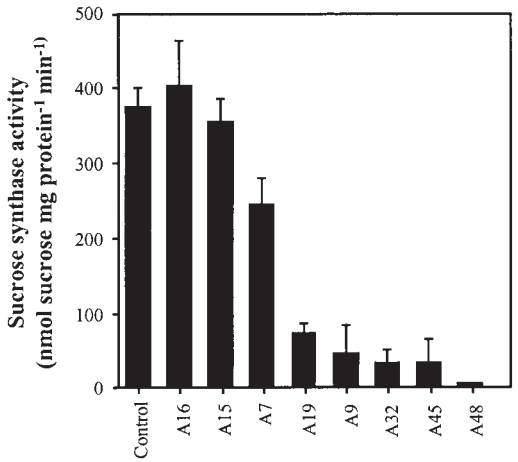

Figure 1. Residual SuSy Activity in Transgenic Tomato Fruits.

SuSy activity was measured in 23-day-old fruits from control and SuSy antisense lines and classified according to the amount of residual activity. Mean values ±SE were calculated from six fruits for each line.

RESULTS

Production of Tomato Plants with Reduced Fruit SuSy Activity

Tomato plants (*Lycopersicon esculentum* cv Summerset) were transformed with a chimeric gene construct consisting of a 538-bp (+46 to +583) antisense fragment of the tomato fruit–specific SuSy cDNA (*TOMSSF*) under the control of the constitutive cauliflower mosaic virus 35S promoter. From the 50 transgenic lines that were obtained, eight, covering the range of SuSy activity from 2 to 107% relative to control (untransformed) fruits, were selected for further analysis (Figure 1). Selection of the eight lines was based on the amount of residual SuSy in the entire fruit; SuSy activity in the control fruits was 375 nmol of sucrose mg protein^{-1} min^{-1}.

To assess the tissue specificity of the inhibition, we also assayed SuSy activity in endosperm and embryo tissues of the plants that had the lowest SuSy activities and compared this with the activity in the control plants. Although the 35S promoter directs a high level of expression in the endosperm and embryo tissues of tomato seeds (D'Aoust et al., 1999), expression of an antisense RNA from *TOMSSF* only slightly inhibited SuSy in the endosperm and did not reduce SuSy activity in the embryo (Table 1). In transgenic lines A48 and A45, which exhibited as much as 98 and 90% SuSy inhibition, respectively, the respective reduction of SuSy activity in the endosperm tissue was only 33 and 20%.

Protein gel blot analysis using polyclonal antibodies raised against maize SuSy (Nguyen-Quoc et al., 1990) on total protein extracts from stems, petioles, and leaves for each line revealed that the amount of SuSy in the transgenic and control lines was identical (Figure 2). In protein extracts from the flowers of antisense plants, however, only a low amount of SuSy was detected (Figure 2). The presence of two immunoreactive bands in the leaf extract reflects the presence of two SuSy isoforms. Indeed, protein gel blot analysis can demonstrate two distinct SuSy isoforms in all analyzed tissues of the tomato plant, including the fruit, depending on the electrophoretic conditions (data not shown). However, the source of the difference between these isoforms has not been resolved.

Carbon Metabolism Gene Expression and Enzyme Activities

RNA gel blot analysis using the 538-bp *TOMSSF* cDNA fragment as a probe revealed that fruit SuSy mRNA content was proportional to the activity for the control plants and lines A9 and A48. However, to reach 107% residual SuSy activity, line A16 massively overexpressed SuSy mRNA (Figure 3, SuSy). The abundance of antisense mRNA fragment was inversely proportional to residual SuSy activity in the transgenic lines and was not detected in the control plants (Figure 3, SuSy). AI mRNA content was reduced in line A16;

Table 1. Comparison of Residual SuSy Activity in the Fruit Pericarp and Seed Tissues of A48, A45, and Untransformed Plants at 23 DAA

	SuSy Activity (nmol Sucrose mg protein^{-1} min^{-1})		
Line	Pericarp[a]	Embryo[b]	Endosperm[b]
A48	7 ± 4	134 ± 16	93 ± 11
A45	35 ± 35	116 ± 4	111 ± 6
Control	375 ± 30	129 ± 14	139 ± 10

[a] Mean ±SE values were calculated from six fruits per line.
[b] Mean ±SE values were calculated from three fruits per line, with a minimum of 20 seeds per fruit.

however, in lines A9 and A48, which had reduced SuSy activity and mRNA content, the AI mRNA content was equal to that of the control plants (Figure 3, AI). The amounts of sucrose phosphate synthase (SPS) and ADP–glucose pyrophosphorylase (AGPase) subunits S1 and B expressed in the different lines analyzed varied, but these variations did not parallel the changes in SuSy activity (Figure 3, SPS, AGPase S1, and AGPase B).

In the transgenic fruit, AI activity increased with decreasing SuSy activity; however, none of the transgenic lines showed a mean AI activity higher than that of the control plants. In the fruits with low SuSy activity (lines A48, A45, A32, A9, and A19), AI activity was equal to that of control plants (Table 2). However, when SuSy activity in the transformed plants was >65% of that of control plants (lines A7, A15, and A16), AI activity decreased to as little as 50% of that of control plants having similar SuSy activity (lines A15 and A16). This decrease in AI activity occurred concomitantly with a decrease in AI mRNA in line A16 (Figure 3, AI). Perhaps the amount of AI mRNA in the pericarp tissues was reduced because of the massive accumulation of SuSy mRNA, suggesting the existence of a coregulation mechanism between the two genes at the transcriptional level. SPS activity, measured under nonlimiting conditions, followed the decrease in SuSy activity but within a much smaller range of variation (Table 2). The lack of correlation between the SPS activity and the amount of mRNA reported above (see Figure 3, SPS) suggests that, in contrast to the effect of the expression of the SuSy antisense RNA on AI activity, post-transcriptional regulation of the enzyme was responsible for the inhibition of SPS when SuSy activity was low.

The decrease in SuSy activity had little or no effect on other major enzyme activities of fruit carbon metabolism. Fructokinase and glucokinase activities were similar in the control and the transgenic plants, regardless of SuSy activity. This is in contrast to the response of potato tubers to antisense SuSy inhibition, in which glucokinase activity increased to 250% of that in control tubers (Zrenner et al., 1995). Variations in AGPase activity could not be attributed to the variations in SuSy activity. As with SuSy, the transgenic plants exhibiting 100% of the AGPase activity of control plants showed a substantial increase in the steady state amount of the AGPase subunit B mRNA. Why line A9 did not overexpress AGPase subunit B mRNA, possibly causing the low activity in that line, is not clear.

Carbohydrate Content and Fruit-Quality Characteristics

Only slight variations in fruit carbohydrate content were measured, except in the line with the least residual SuSy activity, A48. This line exhibited a significant reduction in starch content, possessing 28% less starch than the control fruits ($P = 0.05$). Sucrose and glucose content, however, did not vary significantly with decreasing SuSy activity (Table 3). Fruit-quality characteristics were also evaluated at 23 and 60 DAA to detect a possible role for SuSy activity in determining the physical characteristics of the fruit. At neither 23 nor 60 DAA did the inhibition of SuSy affect the dry weight percentage of the fruit. At 60 DAA, the soluble solids content, expressed as a percentage of the fresh weight, was similar in the SuSy antisense and control plants, even in line A48, in which SuSy activity was reduced to <2% of that in control plants (Table 3). Furthermore, in contrast to the response in potato (Zrenner et al., 1995), the inhibition of SuSy in tomato caused neither a decrease in total protein content nor an alteration of the relative protein accumulation pattern (data not shown).

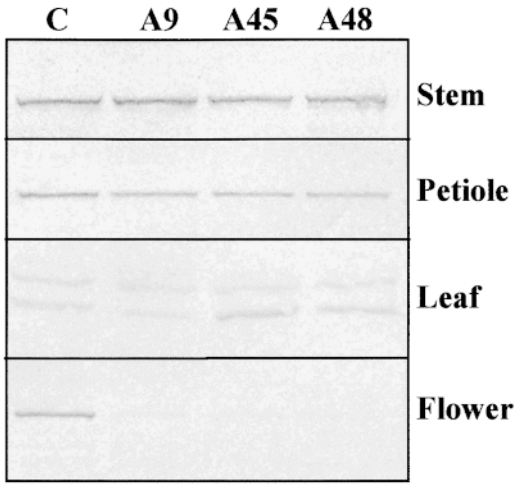

Figure 2. Protein Gel Blot Analysis of SuSy in Different Tissues from Control and Transgenic Lines.

Total proteins were extracted from stems, petals, leaves, and flowers from control plants (C) and from A9, A45, and A48 transgenic lines. Twenty-five micrograms of stem, petiole, and flower proteins or 100 μg of leaf proteins was loaded per lane. SuSy was immunologically detected by using anti–maize SS1 polyclonal antibodies.

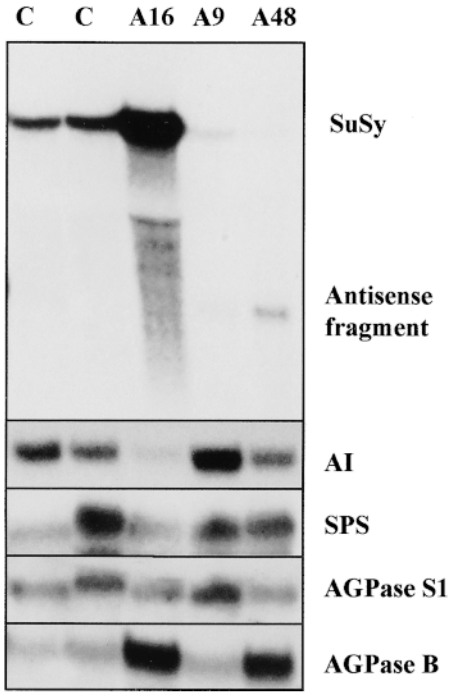

Figure 3. RNA Gel Blot Analysis of Carbon Metabolism in SuSy Antisense Tomato Fruits.

Total mRNA was extracted from 23-day-old fruits of control and A16, A9, and A48 plants. Fifteen micrograms of total fruit RNA was loaded per lane. The RNA gel blot was hybridized with SuSy, AI, SPS, AGPase subunit S1, and AGPase subunit B probes. See Methods for details of the probes. Equal loading of the RNA was ensured by ethidium bromide staining of the gel (data not shown).

In Vitro Sucrose Uptake and Starch Synthesis

The control of carbohydrate import in the sink tissues is probably the most important role suggested for SuSy. To quantify SuSy participation in the control of sucrose import capacity of the fruit, we performed in vitro sucrose uptake experiments with labeled sucrose at 7 and 23 DAA. As shown in Figure 4, fruits from the transgenic lines A9, A45, and A48 had substantially less sucrose unloading capacity at 7 DAA, the amount of imported sucrose in fruits from line A48 being close to 10% of that of the control fruits. By 23 DAA, however, the amount of sucrose being imported by control and transgenic fruits was the same for each (Figure 4B).

To analyze the effect of SuSy inhibition on starch synthesis, we measured the percentage of radioactivity found in the starch fraction after a 1-hr pulse and 2-hr chase. No difference in the amount of newly synthesized starch was found between the control and the transgenic lines. Between 20 and 25% of the imported sucrose was used for starch synthesis in all fruits, irrespective of SuSy activity (Figure 4C).

Fruit Growth Rate and Yields

To determine the profile of fruit growth, we grew eight plants of each control and transgenic line in a greenhouse. Flowers were tagged at anthesis, and fruit diameters were measured at regular intervals from anthesis to maturity. The volume of fruit was estimated from its diameter, considering each fruit to be a perfect sphere. Fruit from the first 4 successive weeks of flowering was used for growth analysis. Figure 5A compares fruit growth profiles between the control plants and the A48 plants for the fruits that developed from the flowers that appeared during the first week of flowering. For this first week, the mean growth rate of the fruits from line A48 was less than that of control fruits. The sizes of the young transgenic fruits from lines A9, A45, and A48 are presented as a percentage of the control fruit volume in Figure 5C, which shows that fruits with SuSy activities between those of A48 (2%) and control (100%) fruits had an intermediate volume at every fruit age.

Table 2. Enzymatic Response to SuSy Inhibition in Tomato Fruits at 23 DAA

	Enzyme Activity (nmol mg protein^{-1} min^{-1})								
		Transgenic Line							
Enzyme	Control	A16	A15	A7	A19	A9	A32	A45	A48
AI[a]	1369 ± 223 (y)	692 ± 161(x)	593 ± 64(x)	630 ± 69 (x)	925 ± 308 (x,y)	1107 ± 251 (x,y)	1415 ± 294 (y)	1397 ± 219 (y)	1370 ± 256 (y)
SPS[a]	69 ± 14 (x,y,z)	80 ± 9 (y,z)	84 ± 7 (z)	83 ± 12 (z)	58 ± 2 (w,x,y,z)	48 ± 2 (w,x)	50 ± 8 (w,x)	57 ± 5 (w,x,y)	45 ± 6 (w)
Fructokinase[b]	48 ± 5	39 ± 3	—[c]	—	—	33 ± 1	—	44 ± 6	40 ± 5
Glucokinase[b]	6.06 ± 0.36	5.18 ± 0.22	—	—	—	6.38 ± 0.27	—	6.53 ± 0.48	7.04 ± 1.43
AGPase[b]	28.35 ± 0.18	29.36 ± 4.55	—	—	—	14.90 ± 0.86	—	26.00 ± 2.50	30.51 ± 2.69

[a] Mean ±SE values were calculated from six fruits. Significant differences between AI or SPS activities are indicated by different letters (w, x, y, or z) in parentheses. Means separation was performed by least squares difference at P = 0.05.
[b] Mean ±SE values were calculated from three fruits.
[c] Not measured.

Table 3. Fruit Carbohydrate Content and Quality Characteristics in SuSy Antisense Tomato Plants

	Carbohydrate Content at 23 DAA (mg g fresh weight^{-1})					
Line	Starch[d]	Sucrose[e]	Glucose[e]	% Dry Weight[a] (23 DAA)	% Dry Weight[b] (60 DAA)	% Soluble Solids[c] (60 DAA)
Control	13.36 ± 1.16 (y)	1.66 ± 0.10	10.36 ± 0.55	6.40 ± 0.14	4.41 ± 0.34	3.65 ± 0.29
A16	15.35 ± 1.49 (y)	2.01 ± 0.26	9.02 ± 0.82	7.04 ± 0.43	4.35 ± 0.44	3.80 ± 0.65
A9	14.53 ± 1.39 (y)	2.08 ± 0.39	11.44 ± 0.48	6.36 ± 0.23	6.34 ± 0.39	3.62 ± 0.52
A45	13.67 ± 1.44 (y)	2.24 ± 0.46	10.87 ± 0.81	6.86 ± 0.57	4.30 ± 0.31	3.49 ± 0.26
A48	9.67 ± 0.73 (x)	2.01 ± 0.34	11.18 ± 0.52	6.49 ± 0.21	4.18 ± 0.23	3.40 ± 0.29

[a] Mean ±SE values were calculated from three to five fruits per line.
[b] Mean ±SE values were calculated from seven to eight fruits per line.
[c] Mean ±SE values were calculated from 10 fruits per line.
[d] Mean ±SE values were calculated from four to five fruits per line. Significant differences between starch content values are indicated by different letters (x or y) in parentheses. Means separation was performed by least squares difference at P = 0.05.
[e] Mean ±SE values were calculated from 11 to 13 fruits per line.

For example, at 7 DAA, the fruits from lines A48, A45, and A9 (with 2, 9.3, and 12.5% SuSy activity, respectively, compared with control fruits) had 25, 35, and 60% of the volume of control fruits, respectively. However, this difference in fruit growth rate disappeared in the fruits that developed from flowers appearing during subsequent weeks because the growth rate of the control fruits decreased to a value similar to that of the A48 fruits. The overall growth of the fruits was not greater in the control lines than in the A48 lines (Figure 5B).

Fruit set was quantified for the SuSy antisense plants and compared with that of the control plants. Inhibition of SuSy was followed by a decrease in fruit setting from 68% in the control plants to 18% in lines A48 and A45 (Figure 6). Interestingly, the first four tagged flowers of the plants from the control and the A48 lines developed into mature fruits (see Figure 6, week 1). Later during plant development, however, a marked difference emerged between the control plants and those with reduced SuSy activity. The proportion of flowers tagged during this period that set fruit drastically dropped from 59% in the control plants to 0% in the plants with 2% SuSy activity (line A48) (see Figure 6, weeks 2 and 3).

Because the number of flowers developing into mature fruits decreased as inhibition of SuSy activity increased, we investigated whether this effect influenced the yields of the plants or instead was counterbalanced by the production of more flowers. When the number of fruits per plant was measured for each line 3 months after the development of the first flowers, the plants from lines A48 and A45 yielded 45 and 60% fewer fruits than did the control plants, respectively (Figure 7A). This reduced number of fruits per plant could not be specifically attributed to a reduction in the number of trusses per plant or to the number of fruits per truss: both showed only a slight difference when analyzed statistically (Figures 7B and 7C).

DISCUSSION

Constitutive Expression of the *TOMSSF* Antisense Fragment Leads to Tissue-Specific Inhibition of SuSy Activity in Tomato

We generated transgenic tomato plants with reduced SuSy activity in fruit by expressing an antisense RNA fragment of the *TOMSSF* gene under the control of the cauliflower mosaic virus 35S promoter. Inhibition of SuSy expression was also detected in the flowers, but little or no inhibition was found in the stem, leaf, and seed tissues. The inhibition of SuSy activity in the flowers is not surprising because the *TOMSSF* cDNA was isolated from tomato pistil mRNAs and is therefore expressed in this tissue as well as in the fruit (Wang et al., 1993b).

Because the coding region of *TOMSSF* is 98.3% similar to that of *Sus4-16*, the tuber-expressed *TOMSSF* gene in potato, it has been proposed that the two genes are homologs (Fu and Park, 1995; Chengappa et al., 1998). This proposition is supported by the expression pattern of a potato *Sus4*–β-glucuronidase (*GUS*) gene construct in transgenic tomato. In these plants, GUS activity was found mainly in flower and fruit tissues (R. Anguenot and B. Nguyen-Quoc, unpublished data). According to Fu and Park (1995), the *Sus3* gene is expressed highly in stems and at a much lower extent in leaves of potato. The tomato *Sus3* mRNA was also found in ripe fruits (Chengappa et al., 1998). In our transgenic plants, the localization of SuSy

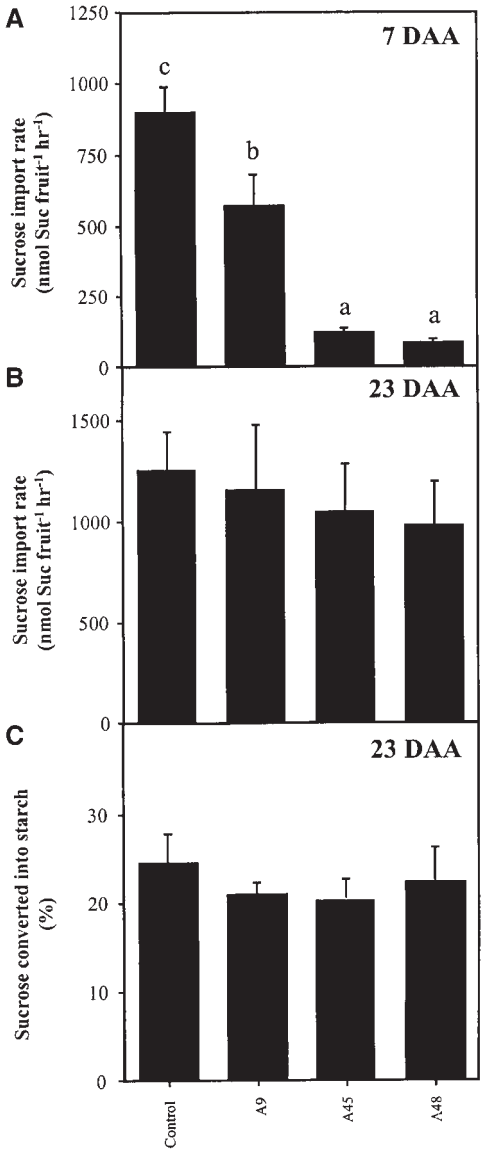

Figure 4. In Vitro Sucrose Import and Starch Synthesis in Antisense Tomato Fruits.

(A) and **(B)** Total sucrose (Suc) unloaded into the fruit at 7 and 23 DAA, respectively. The amount of sucrose was calculated from the amount of radioactivity incorporated after a 1-hr pulse in 60 mM sucrose containing 20 μCi/mL (7 DAA) or 3 μCi/mL (23 DAA) ^{3}H-sucrose. In **(A)**, significant differences between values are indicated by different letters (a, b, or c) over the columns.

(C) Percentage of sucrose converted into starch in 23-day-old fruits after a 2-hr chase period.

Mean values ±SE were calculated from three **(A)** and nine (**[B]** and **[C]**) fruits for each line.

inhibition corresponds with the tissue specificity of *Sus4* (*TOMSSF*), and *Sus3* expression did not decrease in response to the presence of the antisense fragment. Similarly, SuSy activity was slightly or not at all diminished in the seed tissues. Therefore, the inhibition of SuSy that resulted from the constitutive expression of the 538-bp antisense *TOMSSF* fragment appeared to be specific for the *Sus4* (*TOMSSF*) gene products.

SuSy Activity Is Not Essential for Starch Synthesis in Tomato Fruit

In maize, mutations at the *Shrunken1* (*Sh1*) locus are accompanied by a 40% loss of kernel starch content (Chourey and Nelson, 1976). Similarly, in potato, antisense inhibition of tuber SuSy activity to 5% of that of the control plants caused a 34% reduction in starch accumulation and a 50% decrease in tuber dry weight ratio (Zrenner et al., 1995). We did not observe a decrease in tomato fruit dry weight associated with reduced starch content at 23 DAA. Although starch content was reduced in line A48, which exhibits <2% residual SuSy activity in fruit, this reduction in starch content was not caused by a decreased capacity to synthesize starch. First, the fruits from the control lines and from lines A48 and A45 showed similar AGPase activity. Second, regardless of SuSy activity, the percentage of sucrose converted into starch was constantly maintained at 20 to 25%. These results indicate that in the tomato fruit, the use of the SuSy pathway to metabolize newly imported sucrose does not lead to a greater incorporation into starch than when the AI pathway is used. This finding supports the previous demonstration that throughout tomato fruit development, the amount of starch synthesized in the amyloplast is triggered by the amount of sucrose unloaded in the fruit and not by the proportion of sucrose converted to starch (N'tchobo et al., 1999). In contrast, Wang et al. (1993a) suggested that the inhibition of SuSy activity was responsible for the reduced capacity to incorporate sucrose into starch after heat shock treatment. However, heat shock may have affected the production and transport of metabolites needed for starch synthesis inside the amyloplast and thus may have been responsible for the lower capacity to synthesize starch.

The cause of the marked reduction in starch content and dry weight percentage observed in potato and in the upper part of maize endosperm in response to reduced SuSy activity may lie in the low AI activity of these plants (Zrenner et al., 1995; Cheng et al., 1996). Under low AI activity, the decreased SuSy activity caused a significant reduction in total sucrolytic activity of the tissue, resulting in the incapacity of the tuber and the maize kernel to use the incoming sucrose as a source of carbohydrate to sustain downstream carbohydrate metabolism. Comparing our results with those from Zrenner et al. (1995), we found that AI activity of tomato fruit at 23 DAA is 3400-fold that of the potato tuber. Hence, with

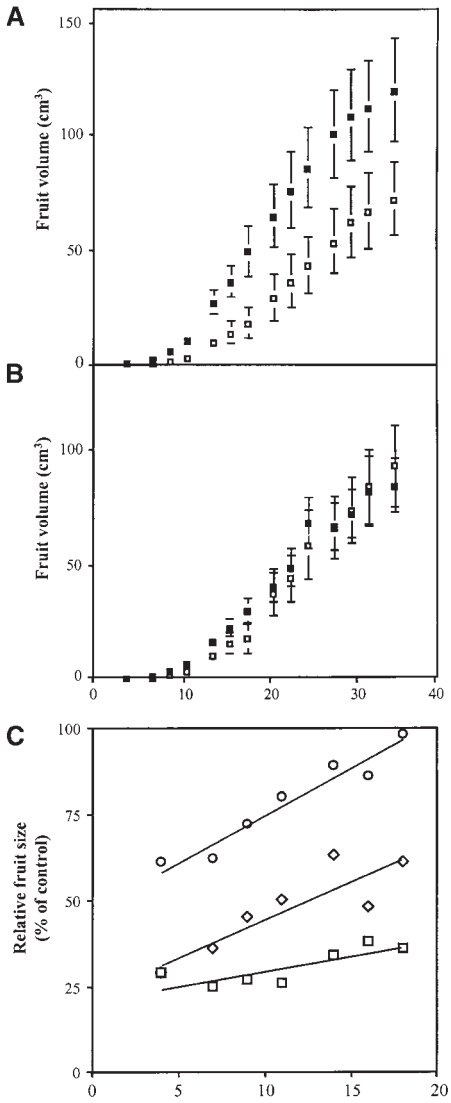

Figure 5. Comparison of Fruit Growth between Line A48 and the Control Line.

(A) Growth profile of the fruits developed from the first week of flowering between 4 and 35 DAA.
(B) Overall growth profile of the fruits between 4 and 35 DAA.
(C) Relative size of SuSy antisense fruits, as a percentage of the control fruit size, between 4 and 18 DAA.
In **(A)** and **(B)**, mean values ±SE were calculated from four to 15 fruits for each line. Filled squares, control plants; open squares, A48; open diamonds, A45; open circles, A9.

its high AI activity, the tomato fruit would still produce the derivatives needed for fruit carbon metabolism. However, the products of sucrose hydrolysis by AI, fructose and glucose, are not phosphorylated. A demand for hexose phosphates in the cytosol, as the result of reduced SuSy activity, may thus stimulate starch degradation, leading to the slight reduction of starch content observed for line A48 at 23 DAA. Because the synthesis of starch was not modified in the transgenic lines, the reduced starch content observed in line A48 may have instead resulted from an increased rate of starch degradation.

Recently, Chourey et al. (1998) attributed the starch deficiency of the *sh1* endosperm in maize to cell degeneration rather than to reduced starch biosynthesis. According to their mutant analysis, they proposed an isoform-specific role for SuSy. SS1 (for SuSy 1, the product of the *Sh1* gene) would be responsible for the production of precursors for cellulose biosynthesis, whereas the SS2 isoform (the product of the *Sus1* gene) would provide the substrates for starch synthesis. Unfortunately, our study of tomato fruit did not allow us to determine such an isoform-specific role for the SuSy activity in the fruit because almost all SuSy activity could be lost by expressing the *TOMSSF* RNA fragment. However, our results indicate that the SuSy activity present in tomato fruit is not essential for starch synthesis. Moreover, the normal growth and final size of fruit from lines A45 and A48 suggest that no cell degeneration is associated with the loss of fruit SuSy isoform(s).

Inhibition of Fruit SuSy Activity Leads to Reduced Unloading Capacity of the Young Fruit

As a result of the observed correlation between SuSy activity and tomato fruit fresh weight (Stommel, 1992; Sun et al., 1992), dry weight (Demnitz-King, 1993), and sucrose import capacity (N'tchobo et al., 1999), SuSy activity has been proposed to control the capacity of the tomato fruit to import

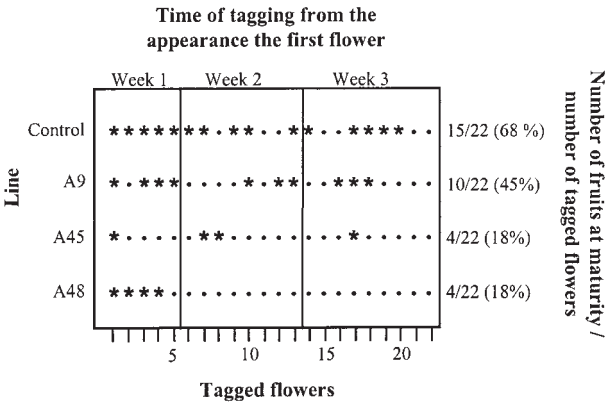

Figure 6. Fruit Set Efficiency in Control and Transgenic Tomato.

Twenty-two flowers distributed on eight plants were tagged for each line. Fruits set during the 3 first weeks of flowering (weeks are separated vertically into three distinct blocks in the diagram) are represented as aborted (dots) or grown to maturity (asterisks).

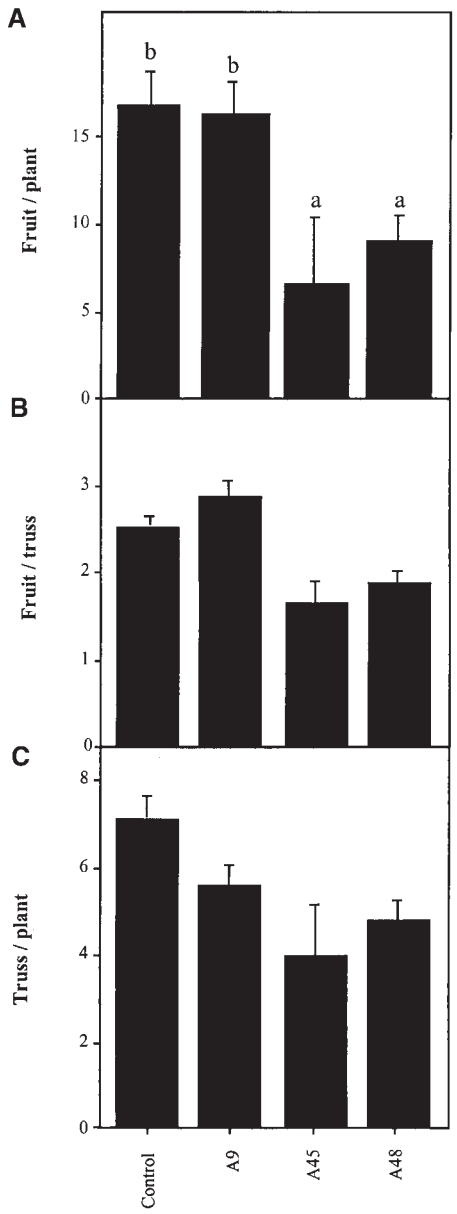

Figure 7. Productivity of the SuSy Antisense Tomato Plants.

(A) and **(B)** Yields are expressed in fruits per plant **(A)** or in fruits per truss **(B)**.

(C) Yields expressed in trusses per plant.

Mean values ±SE were calculated from three to eight plants for each line. Significant differences between values are indicated by different letters (a or b). Means separation was performed by least squares difference at P = 0.05.

and metabolize sucrose. The transgenic lines containing the lowest SuSy activity also showed the most diminished capacity to import ^{3}H-sucrose in 7-day-old fruits. This result indicates that SuSy activity controls the capacity of the young tomato fruit to metabolize sucrose. However, later in development, even when SuSy activity is greatest (20 to 25 DAA), the sucrose-metabolizing capacity of the fruit is not dependent on SuSy activity because the in vitro uptake of ^{3}H-sucrose by the fruits at 23 DAA was not reduced by 98% inhibition of SuSy activity.

In the absence of SuSy activity, the main pathway for sucrose metabolism in the fruit cells was the vacuolar AI pathway. Hence, young (7 DAA) transgenic fruits lacking SuSy activity relied for their supply of carbohydrate on the activity of sucrose transporters in the tonoplast and on AI activity. Because young fruit (0 to 14 DAA) mainly develops through cell division (Ho, 1988) and because vacuoles are restricted in size during cell division, the sucrose-metabolizing capacity of young SuSy antisense fruits was well less than that of control fruits and older antisense fruits (14 to 55 DAA), which are composed of cells containing large vacuoles with high AI activity (Yelle et al., 1991).

In vivo, the reduced sucrose import capacity of transgenic fruits caused the growth rate to diminish for the first fruits of each plant. This reduction was particularly important for very young fruits. Together with the in vitro observations, this finding suggests that SuSy activity controls the unloading kinetics of the very young tomato fruit and that the unloading kinetics of the young fruit determines the growth rate in the conditions in which the first fruits develop. Fisher (1977), Bertin and Gary (1993), and Bertin (1995) suggested that when the first fruits develop on a plant, the competition for assimilates is low, and that the production of sucrose by the source leaves is higher than the demand for sucrose by the fruits. Furthermore, according to a mechanistic model of phloem transport developed by Minchin et al. (1993), the sucrose-metabolizing capacity of these fruits is saturated. Under such conditions, the growth rate of the fruits would be limited by their unloading kinetics (sink limited). Effectively, the model predicts that any increase in sucrose production by the source leaves would lead to an equivalent increase in sucrose concentration near the sink (the unloading process being already saturated), without changing the concentration gradient, which is the driving force of the phloem transport according to the bulk flow theory of Minchin et al., 1993.

The effect of SuSy inhibition on fruit growth became negligible as the number of fruits borne by the tomato plants increased. Subsequent control fruits were smaller than those from the first week of flowering, and overall, the mean volume of the transgenic fruits was equal to that of the control fruits at any stage of development. This result can be explained by an increase in the competition for photoassimilates, given the increasing numbers of fruits, which would have led to a decreased sucrose concentration in the phloem. Under such limiting substrate conditions, SuSy activity would not have determined the rate of fruit growth.

SuSy Activity Determines Fruit Set and Productivity

Concomitant with the inhibition of SuSy activity in the flowers and fruit tissue, we observed an important reduction of fruit set in lines A9, A45, and A48. Fruit set is well known to be resource limited (Stephenson, 1981), and it has been demonstrated that with the increasing number of fruits borne by a plant, competition for the photoassimilates becomes higher and the number of fruits able to develop to maturity decreases (Picken, 1984; Bertin, 1995). Similarly, a decrease in the photosynthetic production of sucrose related to pruning or shading the leaves causes a proportional decrease in fruit set (Leopold and Scott, 1952). The results presented here show not only that fruit set is resource limited, but also that SuSy activity, by controlling their unloading capacity, determines the capability of the fruits to set properly. The detrimental effect of SuSy inhibition on fruit set had repercussions on the productivity of the plants. Effectively, an important decrease in the number of fruits produced by a mature plant was observed in the transgenic lines containing $<10\%$ residual fruit SuSy.

Given the results presented here, we suggest that SuSy activity determines the capacity of the very young fruit in metabolizing sucrose from the phloem. For the first fruits of the plant, because the competition for sucrose is low, the concentration is saturating for the unloading kinetics of the fruit, and SuSy activity determines the amount of carbohydrate imported into the fruit. Later during the development of the plant, the competition for photoassimilates increases with the number of developing fruits, and the concentration of sucrose in the phloem decreases. For these fruits, SuSy activity influences the competitiveness of importing and metabolizing sucrose and hence determines their setting ability.

METHODS

Plant Material

Tomato (*Lycopersicon esculentum* cv Summerset) seeds were purchased locally (Gerard Bourbeau et Fils Inc., Charlesbourg, Canada). In vitro–grown plants were cultivated on Murashige and Skoog medium (Murashige and Skoog, 1962) in a culture room with 16 hr of light (at 25°C) and 8 hr of darkness (at 22°C). The irradiance was 450 μmol m^{-2} sec^{-1} with 50% relative humidity. For fruit analysis, six to eight plants were multiplied by cutting and grown under greenhouse conditions with 50% relative humidity and supplemented irradiance of 300 μmol m^{-2} sec^{-1} under the same light photoperiod and temperature regimen. The flowers were tagged at anthesis to determine fruit age.

Sucrose Synthase Antisense Gene Construction

The sucrose synthase (SuSy) cDNA fragment from tomato was amplified by using reverse transcription–polymerase chain reaction (RT-PCR), with primer design based on the tomato fruit sucrose synthase sequence cloned by Wang et al. (1993b) (GenBank accession number L19762). The oligonucleotide sequences used to amplify 538 bp ($+46$ to $+584$) from the *SuSy* cDNA, including the start codon (in italics), were as follows: SST-5, 5′-CTGCTGAATCAACTATA*ATG*G-CTGAAC-3′; and SST-3, 5′-GCTTTCCTTGTCATGGAACATTTTG-3′. A coupled one-step RT-PCR method described by Aatsinki et al. (1994) was used to amplify the tomato fruit *SuSy* fragment. The amplified fragment was subcloned into the pCRII cloning vector (Invitrogen, San Diego, CA) and then inserted downstream of the cauliflower mosaic virus 35S constitutive promoter in the binary vector pBI121 (Clonetech Laboratories, Palo Alto, CA) after deletion of the β-glucuronidase (*GUS*) gene between the SacI and XbaI sites.

Plant Transformation

Tomato plants were transformed essentially as described previously (D'Aoust et al., 1999). Briefly, well-expanded cotyledons of 10-day-old seedlings were excised and placed upside down on the induction medium. After 3 days of precultivation, green cotyledons swelling in size were cut transversally and transferred into a diluted *Agrobacterium tumefaciens* culture for 15 min. After 3 days of cocultivation, the cotyledons were placed upside down onto the selection medium. After 3 weeks in the selection medium, the calli were excised from the cotyledons and transferred to the organogenesis medium. The shoots grown from the calli were screened for enhanced ability to form roots on kanamycin-containing medium. The incorporation of the transgene and the independence of the transgenic lines were monitored by DNA gel blot analysis (data not shown).

DNA and RNA Extractions

Genomic DNA for DNA gel blot analysis was prepared by using cetyltrimethylammonium bromide, as described by Richards et al. (1998). Total RNA was isolated by using the hot phenol extraction method described by De Vries et al. (1988).

RNA Gel Blot Analysis

RNA gel blot analyses were based on the method described in Goldberg (1980). Fifteen micrograms of total RNA was separated by denaturating agarose gel electrophoresis. The RNA was transferred overnight to a nylon membrane (Boehringer Mannheim) with 10 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl and 0.015 M sodium citrate) containing 2% (w/v) formaldehyde. After transfer, membranes were placed for 1 min under UV light for RNA fixation. The hybridization was performed at 42°C in the presence of 50% formamide, and the last washing steps were performed with 0.2 $\times$ SSC and 0.1% (w/v) SDS at 60°C. The membranes were autoradiographed at -70°C with use of Kodak Biomax films (Kodak, New Haven, CT). The cDNA clones labeled by random priming and used as probes were as follows: SuSy, the *TOMSSF* fragment used for the antisense construct (see Sucrose Synthase Antisense Gene Construction, above, for details); acid invertase (AI), the *TIV1* cDNA (GenBank accession number M81081) described by Klann et al. (1992); and sucrose phosphate synthase (SPS), an RT-PCR fragment that was amplified from tomato leaf total RNA with use of the following primers: SPSD-5, 5′-AAGGAT-CCATGCTGATAATACATTAGACCCCGAAG-3′; and SPSD-3, 5′-TAT-CACCACTTTCTCCGACGAAAAC-3′, based on the potato SPS

sequence (EMBL accession number X73477). Sequencing of the fragment obtained revealed 96% identity with the potato SPS sequence. The probes for AGPase small subunit (B) and large subunit (S1) were cDNA fragments PCR-amplified with forward and reverse primers from the pBB and pBS1 plasmids, respectively (Chen et al., 1997).

Protein Gel Blot Analysis

Liquid nitrogen–frozen plant tissues were ground, and the proteins from 250 mg of powder were extracted on ice for 30 min with four volumes of extraction buffer (50 mM Hepes, pH 7.0, 5% [v/v] ethylene glycol, 1% [w/v] polyvinylpolypyrrolidone, 20 mM β-mercaptoethanol, 10 mM $MgCl_2$, 1 mM EDTA, 2 mM DTT, and 1 mM phenylmethylsulfonyl fluoride). After centrifugation for 10 min at 20,000*g* at 4°C, the supernatant was collected and precipitated on ice for 15 min with 2.5% trichloroacetic acid. The pellet was harvested after 15 min of centrifugation at 6000*g* at 4°C and resuspended in loading buffer (400 mM Tris, pH 6.8, 10% SDS, 10% glycerol, 1.4 M β-mercaptoethanol, and 0.1 mM bromophenol blue) to a final concentration of 5 μg/μL (stem, petiole, and flowers) or 10 μg/mL (leaf). Before loading, 1 M Tris was added until the extract regained its blue color (~10 μL). Protein samples (25 μg per lane for stem, petiole, and flower extracts and 100 μg for the leaf extract) were separated by 10% SDS-PAGE and blotted onto nitrocellulose membranes. The immunological detection of SuSy was performed by using anti-SS1 polyclonal antibodies (Nguyen-Quoc et al., 1990) diluted 1:5000. Alkaline phosphatase–coupled anti–rabbit IgG antibodies were used to reveal the presence of the primary antibodies.

Enzyme Activity Analysis

Fresh fruit tissues harvested 23 days after anthesis (DAA) were ground to powder with liquid nitrogen and homogenized in extraction buffer (200 mM Hepes, pH 7.0, 10% [v/v] ethylene glycol, 1% [w/v] polyvinylpolypyrrolidone, 20 mM β-mercaptoethanol, 10 mM $MgCl_2$, 1 mM EDTA, 2 mM DTT, and 1 mM phenylmethylsulfonyl fluoride) with a sample-to-buffer ratio of 1:5 (w/v). The homogenate was centrifuged for 5 min at 20,000*g* at 4°C, and the supernatant was desalted by Sephadex G-25 gel filtration before determining the enzyme activities. SuSy (Huber and Akazawa, 1986), invertase (Klann et al., 1993), SPS (Huber and Huber, 1991), glucokinase, and fructokinase (Huber and Akazawa, 1986) activities were assayed as described. AGPase activity was assayed by measuring the formation of NADH at 340 nm (25°C) in a reaction mixture composed of 40 mM Hepes, pH 7.5, 10 mM $MgCl_2$, 2 mM DTT, 0.4 mM NAD, 1 mM ADP-glucose, 8 units/mL phosphoglucomutase, and 4 units/mL glucose-6-phosphate dehydrogenase. The reaction was initiated by adding 2 mM PPi to the mixture.

Starch and Soluble Sugars Quantification

Fresh fruit tissues were frozen in liquid nitrogen and ground to powder. The powder (300 mg) was extracted with 1 mL of 80% (v/v) ethanol. The supernatant was used to determine glucose and sucrose content with a SELECT biochemistry analyzer (model 2700; Yellow Spring Instrument Co., Yellow Spring, OH), as previously described by Nguyen-Quoc et al. (1999). The pellet was washed twice with 1 mL of 80% (v/v) ethanol and dried at 80°C for 15 min. Starch was solubilized in 800 μL of 0.02 M NaOH at 100°C for 10 min and neutralized by the addition of 200 μL of 1.5 M citrate buffer, pH 4.4. The solubilized starch was hydrolyzed overnight at 25°C after the addition of 150 units/mL amylase and 150 units/mL amyloglucosidase. The glucose released was quantified by using the SELECT biochemistry analyzer.

Determination of Soluble Solids Content and Dry Weight Percentage

Mature fruit was harvested at 60 DAA and homogenized. The resulting juice was centrifuged at 5000*g* for 15 min, and the supernatant was used for the determination of soluble solids with a hand refractometer (Fisher Scientific Ltd., Nepean, Canada). For dry weight determination, 5 mL of the homogenate from each fruit was weighed, dried to constant weight at 60°C, and weighed again.

[Fructose-1-^{3}H(N)]-Sucrose Uptake by Detached Fruits

The determination of sucrose uptake into fruits was performed as previously described (N'tchobo et al., 1999). Fruits were separated from the plant by cutting the pedicel 3 cm away from the fruit, and the pedicel was then buried in a solution containing 60 mM sucrose with either 20 μCi/mL (7 DAA) or 3 μCi/mL (23 DAA) [fructose-1-^{3}H(N)]-sucrose (Du Pont–NEN Research Products, Mississauga, Canada). After a 1-hr pulse, sucrose unloading into the 7-day-old fruit was stopped, and the fruit was frozen in liquid nitrogen. For 23 DAA, the fruit was fed with an equal concentration of nonradioactive sucrose for a 2-hr chase. After the chase period, the pedicel was removed, and the fruit was frozen in liquid nitrogen. After grinding the tissue, we measured the radioactivity incorporated by using a liquid scintillation counter (model 1409; Wallac Oy, Turku, Finland). The determination of radioactivity incorporated into starch was performed by extracting the starch, as described above, and measuring the radioactivity in the starch fraction by using a liquid scintillation counter.

Statistical Analysis

Statistical analysis was performed with the Fisher's protected least squares difference procedure of the SuperANOVA version 1.11 application (Abacus Concepts, Inc., Berkeley, CA).

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REFERENCES

Aatsinki, J.T., Lakkakorpi, J.T., Pietilä, E.M., and Rajaniemi, H.J. (1994). A coupled one-step reverse transcription PCR procedure for generation of full-length open reading frames. BioTechniques **16,** 282–288.

Avigad, G. (1982). Sucrose and other disaccharides. In Encyclopedia of Plant Physiology, F.A. Loewus and W. Tanner, eds (Heidelberg, Germany: Springer-Verlag), pp. 217–347.

Bertin, N. (1995). Competition for assimilates and fruit position affect fruit set in indeterminate greenhouse tomato. Ann. Bot. **75,** 55–65.

Bertin, N., and Gary, C. (1993). Tomato fruit-set: A case study for validation of the model TOMGRO. Acta Hort. **328,** 185–193.

Chen, B.Y., Janes, H.W., and Gianfagna, T. (1997). Expression of tomato ADP-glucose pyrophosphorylase in different tissues. Plant Physiol. **114** (suppl.), 54 (abstr).

Cheng, W.H., Taliercio, E.W., and Chourey, P.S. (1996). The *Miniature1* seed locus of maize encodes a cell wall invertase required for normal development of endosperm and maternal cells in the pedicel. Plant Cell **8,** 971–983.

Chengappa, S., Loader, N., and Shields, R. (1998). Cloning, expression and mapping of a second tomato sucrose synthase gene, *Sus3* (accession nos. AJ011319 and AJ011534). Plant Physiol. **118,** 1533.

Chourey, P.S., and Nelson, S.E. (1976). The enzymatic deficiency conditioned by the *shrunken-1* mutation in maize. Biochem. Genet. **14,** 1041–1055.

Chourey, P.S., Taliercio, E.W., Carlson, S.J., and Ruan, Y.L. (1998). Genetic evidence that the two isozymes of sucrose synthase present in developing maize endosperm are critical, one for cell wall integrity and the other for starch biosynthesis. Mol. Gen. Genet. **259,** 88–96.

Craig, J., Dejardin, A., Handley, L., Hedley, C.L., Wang, T.L., and Smith, A.M. (1996). A mutation that affects sucrose synthase in pea alters the C and N metabolism of seeds and nodules. Plant Physiol. **111** (suppl.), 139 (abstr).

Dali, N., Michaud, D., and Yelle, S. (1992). Evidence for the involvement of sucrose phosphate synthase in the pathway of sucrose accumulation in sucrose accumulating tomato fruits. Plant Physiol. **99,** 434–438.

Damon, S., Hewitt, J., Nieder, M., and Bennett, A.B. (1988). Sink metabolism in tomato fruit. II. Phloem unloading and sugar uptake. Plant Physiol. **87,** 731–736.

D'Aoust, M.A., Nguyen-Quoc, B., and Yelle, S. (1999). Upstream regulatory regions from the maize *Sh1* promoter confer tissue-specific expression of the *GUS* gene in tomato. Plant Cell Rep. **18,** 803–808.

Demnitz-King, A.C. (1993). Sucrose Metabolism in Relation to Import and Compartmentation of Carbohydrates in Developing Tomato Fruit (*Lycopersicon* spp.). PhD Dissertation (Wye, UK: London University).

De Vries, S., Hoge, H., and Bisseling, T. (1988). Isolation of total and polysomal RNA from plant tissues. In Plant Molecular Biology Manual, S.T. Gelvin, R.A. Schilperoort, and D.P.S. Verma, eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 1–13.

Fisher, K.J. (1977). Competition effects between fruit trusses of the tomato plant. Sci. Hort. **7,** 37–42.

Fu, H., and Park, W.D. (1995). Sink- and vascular-associated sucrose synthase functions are encoded by different gene classes in potato. Plant Cell **7,** 1369–1385.

Goldberg, D.A. (1980). Isolation and partial characterization of the *Drosophila* alcohol dehydrogenase gene. Proc. Natl. Acad. Sci. USA **77,** 5794–5798.

Hawker, J.S. (1985). Sucrose. In Biochemistry of Storage Carbohydrate in Green Plants, P.M. Dey and R.A. Dixon, eds (London: Academic Press), pp. 1–51.

Ho, L.C. (1988). Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Annu. Rev. Plant Physiol. Plant Mol. Biol. **39,** 355–378.

Huber, S.C., and Akazawa, T. (1986). A novel sucrose synthase pathway for sucrose degradation in cultured sycamore cells. Plant Physiol. **81,** 1008–1013.

Huber, S.C., and Huber, J.L. (1991). Regulation of maize leaf sucrose phosphate synthase by protein phosphorylation. Plant Cell Physiol. **32,** 319–326.

Klann, E., Yelle, S., and Bennet, A.B. (1992). Tomato fruit acid invertase complementary DNA. Plant Physiol. **99,** 351–353.

Klann, H.M., Chetelat, R.T., and Bennet, A.B. (1993). Expression of acid invertase gene controls sugar composition in tomato (*Lycopersicon*) fruit. Plant Physiol. **103,** 863–870.

Klann, H.M., Hall, B., and Bennett, A.B. (1996). Antisense acid invertase (*TIV1*) gene alters soluble sugar composition and size in transgenic tomato fruit. Plant Physiol. **112,** 1321–1330.

Leopold, A.C., and Scott, F.I. (1952). Physiological factors in tomato fruit-set. Am. J. Bot. **39,** 310–317.

Minchin, P.E.H., Thorpe, M.R., and Farrar, J.F. (1993). A simple mechanistic model of phloem transport which explains sink priority. J. Exp. Bot. **44,** 947–955.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. **15,** 473–497.

Nguyen-Quoc, B., Krivitzki, M., Huber, S.C., and Lecharny, A. (1990). Sucrose synthase in developing maize leaves. Regulation of activity by protein level during the import to export transition. Plant Physiol. **94,** 516–523.

Nguyen-Quoc, B., N'tchobo, H., Foyer, C., and Yelle, S. (1999). Overexpression of sucrose phosphate synthase increases sucrose unloading in transformed tomato fruit. J. Exp. Bot. **335,** 785–791.

N'tchobo, H., Dali, N., Nguyen-Quoc, B., Foyer, C.H., and Yelle, S. (1999). Starch synthesis in tomato remains constant throughout fruit development and is dependent on sucrose supply and sucrose synthase activity. J. Exp. Bot. **50,** 1457–1463.

Ohyama, A., Ito, H., Sato, T., Nishimura, S., Imai, S., and Hira, M. (1995). Suppression of acid invertase activity by antisense RNA modifies the sugar composition of tomato fruit. Plant Cell Physiol. **36,** 369–376.

Picken, A.J.F. (1984). A review of pollination and fruit set in the tomato (*Lycopersicon esculentum* Mill.). J. Hort. Sci. **59,** 1–13.

Richards, E., Reichardt, M., and Rogers, S. (1998). Preparation of plant DNA using CTAB. In Current Protocol in Molecular Biology, F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K.S. Struhl, eds (Boston: Current Protocols Publishers), pp. 2.3.3–2.3.7.

Ruan, Y.-L., and Patrick, J.W. (1995). The cellular pathway of postphloem sugar transport in developing tomato fruit. Planta **196,** 434–444.

Skot, L., Gordon, A.J., Timms, E., James, C.L., Webb, K.J., and Mizen, S. (1997). Down-regulation of sucrose synthase expression and activity in transgenic hairy roots of *Lotus japonicus.* Symbiosis **22,** 241–254.

Stephenson, A.G. (1981). Flowers and fruit abortion: Proximate causes and ultimate functions. Annu. Rev. Ecol. Syst. **12,** 253–279.

Stommel, J.R. (1992). Enzymic components of sucrose accumulation in the wild tomato species *Lycopersicon peruvianum.* Plant Physiol. **99,** 324–328.

Sun, J., Loboda, T., Sung, S.-J.S., and Black, C.C.J. (1992). Sucrose synthase in wild tomato, *Lycopersicon chmielewskii*, and tomato fruit sink strength. Plant Physiol. **98,** 1163–1169.

Wang, F., Sanz, A., Brenner, M.L., and Smith, A. (1993a). Sucrose synthase, starch accumulation, and tomato fruit sink strength. Plant Physiol. **101,** 321–327.

Wang, F., Smith, A.G., and Brenner, M.L. (1993b). Isolation and sequencing of tomato fruit sucrose synthase cDNA. Plant Physiol. **103,** 1463–1464.

Yelle, S., Hewitt, J.D., Robinson, N.L., Damon, S., and Bennet, A.B. (1988). Sink metabolism in tomato fruit. III. Analysis of carbohydrate assimilation in a wild species. Plant Physiol. **87,** 737–740.

Yelle, S., Chetelat, R.T., Dorais, M., DeVerna, J.W., and Bennet, A.B. (1991). Sink metabolism in tomato fruit. IV. Genetic and biochemical analysis of sucrose accumulation. Plant Physiol. **95,** 1026–1035.

Zrenner, R., Salanoubat, M., Willmitzer, L., and Sonnewald, U. (1995). Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). Plant J. **7,** 97–107.