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Effects of Elevated Sucrose-Phosphate Synthase Activity on Photosynthesis, Assimilate Partitioning, and Growth in Tomato (*Lycopersicon esculentum* var UC82B)

Nathalie Galtier, Christine H. Foyer*, Joan Huber, Toni A. Voelker, and Steven C. Huber

Laboratoire du Métabolisme, Institut National de la Recherche Agronomique, Route de St-Cyr, 78026 Versailles cedex, France (N.G., C.H.F.); Department of Crop Science (J.H.) and United States Department of Agriculture, Agriculture Research Service (S.C.H.), North Carolina State University, Box 7631, Raleigh, North Carolina 27607; and Calgene, Inc., 1920 Fifth Street, Davis, California 95616 (T.A.V.)

The expression of a sucrose-phosphate synthase (SPS) gene from maize (*Zea mays*, a monocotyledon) in tomato (*Lycopersicon esculentum*, a dicotyledon) resulted in marked increases in extractable SPS activity in the light and the dark. Diurnal modulation of the native tomato SPS activity was found. However, when the maize enzyme was present the tomato leaf cells were unable to regulate its activation state. No detrimental effects were observed and total dry matter production was unchanged. However, carbon allocation within the plants was modified such that in shoots it increased, whereas in roots it decreased. There was, therefore, a change in the shoot:root dry weight ratio favoring the shoot. This was positively correlated with increased SPS activity in leaves. SPS was a major determinant of the amount of starch in leaves as well as sucrose. There was a strong positive correlation between the ratio of sucrose to starch and SPS activity in leaves. Therefore, SPS activity is a major determinant of the partitioning of photosynthetically fixed carbon in the leaf and in the whole plant. The photosynthetic rate in air was not significantly increased as a result of elevated leaf SPS activity. However, the light- and CO_2-saturated rate of photosynthesis was increased by about 20% in leaves expressing high SPS. In addition, the temporary enhancement of the photosynthetic rate following brief exposures to low light was increased in the high SPS plants relative to controls. We conclude that the level of SPS in the leaves plays a pivotal role in carbon partitioning. Furthermore, high SPS levels have the potential to boost photosynthetic rates under favorable conditions.

SPS catalyzes the penultimate step of sucrose biosynthesis in leaves (Pontis, 1977). This rate-limiting, allosteric enzyme forms a major control point subject to regulation by metabolites and by covalent modification involving protein phosphorylation (Harbron et al., 1981; Huber, 1983; Stitt et al., 1987; Huber et al., 1989; Stitt and Quick, 1989). The activity of this enzyme can be a limiting factor for de novo Suc synthesis and also photosynthesis (Pontis, 1977; Huber, 1983; Stitt et al., 1987). The amount of extractable SPS activity is known to vary in response to light/dark transitions (Huber et al., 1987) and to the metabolic status of the leaf tissue in terms of substrate concentrations (Harbron et al., 1981; Huber, 1983; Kalt-Torres and Huber, 1987; Kerr and Huber, 1987; Stitt et al., 1987).

Multiple forms of SPS have been observed in leaves of plants such as maize (Kalt-Torres et al., 1987). The multiplicity of enzyme forms appears to arise from posttranslational modifications (Huber et al., 1989; Huber and Huber, 1991) to the enzyme protein because the cDNA isolated from maize appears to have only one predominant species of transcript (Worrell et al., 1991). The maize leaf SPS has been shown to be subject to light/dark regulation (Kalt-Torres and Huber, 1987), a process mediated by phosphorylation of the enzyme protein (Huber and Huber, 1991; Huber et al., 1991). It has been shown that the generation of one type of subunit (molecular mass 135 kD) is sufficient to assemble the SPS holoenzyme in *Escherichia coli* (Worrell et al., 1991).

Transgenic tomato (*Lycopersicon esculentum*) plants expressing both the native SPS and also the SPS gene from maize were produced by Worrell et al. (1991). The active enzyme was expressed from cloned cDNA under the control of the promoter for the gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase from tobacco (*Nicotiana tabacum*) (Worrell et al., 1991). When the maize SPS was expressed in tomato, the differences in enzyme activity between light and dark were much smaller (Worrell et al., 1991) and high levels of SPS activity were always present. This fact may provide an explanation for the perturbations in carbon metabolism observed in these plants (Worrell et al., 1991). For example, increased activities of SPS were shown to modify assimilate partitioning in the leaf (Worrell et al., 1991).

We have studied the metabolic and physiological effects of the expression of the maize gene in the next generation of tomato plants (the T_1 generation, grown from seed). These plants have provided a unique opportunity to investigate the role of SPS in the control of photosynthesis, leaf carbon metabolism, and growth. We propose that SPS activity is a key determinant in assimilate partitioning in leaves.

MATERIALS AND METHODS

Independent studies on the physiology and metabolism of the transgenic and untransformed control tomato (*Lycopersicon esculentum* var UC82B) plants were carried out in Ver-

* Corresponding author; fax 33–30–83–30–96.

Abbreviations: Fru6P, fructose 6-phosphate; Glc6P, glucose 6-phosphate; SPS, sucrose-phosphate synthase; UDPGlc, UDP glucose.

sailles, France, and Raleigh, NC. In the present work, results from both studies are presented.

Plant Material

Seed of transgenic and nontransgenic tomato was obtained from the Roussel Agri-Vet Company (France) and from Calgene, Inc. (Davis, CA). Plants were grown in a glasshouse in Versailles with supplemented lighting and in a growth chamber (Raleigh) with a 14-h photoperiod, 25°C day/22°C night regimen; irradiance was 400 to 700 μmol s^{-1} m^{-2}. Mature adult leaves were used in all experiments reported here.

Photosynthesis Measurements

The CO_2 assimilation rates of whole attached leaves in air were measured using an IR gas analysis apparatus (Analytical Development Company Ltd., UK). CO_2-dependent O_2 evolution from leaf segments was measured in saturating CO_2 in the leaf disc oxygen electrode (Hansatech, UK). Irradiance was varied using neutral density filters.

Immunoprecipitation

Desalted leaf extracts were preincubated with maize-specific monoclonal antibodies (Bruneau et al., 1991). SPS-antibody complexes were precipitated with a commercial preparation of fixed *Staphylococcus aureus* cells (Immunoprecipitin, BRL) as previously described (Walker and Huber, 1989). SPS activity in the supernatant remaining after precipitation of this complex was measured.

SPS Extraction and Assay

In Versailles, leaves were harvested in the glasshouse at midday or following 16 h of darkness. They were plunged immediately into liquid nitrogen and stored at −80°C until required. Leaf material was ground at a ratio of 1 g fresh weight to 3 mL of extraction medium, which consisted of 0.2 M Tris-HCl (pH 8.2), 20 mM $MgCl_2$, 5 mM EDTA, 10 mM DTT, 20% glycerol, and 1 mg mL^{-1} of BSA. The extracts were filtered and centrifuged at 12,000*g* in an Eppendorf centrifuge for 5 min. The soluble fraction was passed through Sephadex G-25M (PD10 columns, Pharmacia) equilibrated with the reaction buffer consisting of 50 mM Hepes-KOH (pH 7.5), 10 mM $MgCl_2$, 2 mM EDTA, and 2 mM DTT. SPS was assayed by the incorporation of [UDP-^{14}C]Glc as described by Salerno et al. (1979), except that the reaction medium consisted of 50 mM Hepes-KOH buffer (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 10 mM Fru6P, and 10 mM [UDP-^{14}C]-Glc (770 Bq mol^{-1}). In Raleigh, leaves were harvested and SPS assayed as described previously (Huber and Huber, 1991). It is important to note that depending on the location, SPS activity was measured under different assay conditions and plants were grown under very different conditions. Nevertheless, the results are remarkably consistent and corroborative in all cases.

Suc, Sugars, Starch, and Metabolite Assays

Unless otherwise indicated on the figures or tables, leaves were harvested at noon in the light or after 16 h of darkness and plunged immediately into liquid nitrogen. The frozen samples were ground in $HClO_4$ (1 M).

Suc, Glc, and Fru were measured enzymically after centrifugation and neutralization of the supernatants with K_2CO_3. Extraction of soluble sugars by this method produced results identical to those obtained by immersion of the tissues in 80% ethanol at 85°C for 1 h and had the advantage of producing a relatively colorless supernatant. Glc, Fru, and Suc were measured enzymically in a reaction mixture consisting of 100 mM Hepes-KOH buffer (pH 7.0), 5 mM $MgCl_2$, 0.8 mM NADP, 1 mM ATP, Glc6P dehydrogenase (2 units), phosphoglucose isomerase (4 units), hexokinase (4 units), and invertase (7000 units) in a total volume of 1 mL. All enzymes were obtained from Boehringer Mannheim. Reduction of NADP was followed at 340 nm. The pellets produced by centrifugation of the extracts used for soluble sugar analysis were subsequently used for the measurement of starch. The pellets were extracted in 10 mL of 80% acetone and centrifuged. The pellets were then dried and resuspended in 4 mL of H_2O and boiled in a water bath at 100°C for 1 h. Starch concentrations were then measured by incubating the pellet fractions in 50 mM sodium acetate buffer (pH 4.6) and 3 units of amylase and 60 units of amyloglucosidase (Boehringer Mannheim). The mixtures were incubated for 1 h at 50°C and aliquots were removed to measure the Glc produced enzymically as above.

Metabolite measurements were made using standard enzymic procedures after neutralization of the samples in K_2CO_3 as described previously (Stitt et al., 1980; Furbank and Foyer, 1986).

Analysis of Shoot:Root Ratios

Plants were harvested 6 weeks after sowing. In all cases the plants were divided into shoots and roots and these were dried in an oven at 60°C for 48 h and then weighed. The average dry weight of the whole plant was 10 g.

RESULTS

Comparison of Activities of the Enzyme between Controls and Transgenics

In Versailles and Raleigh, we examined 10 lines of tomato transformants that showed different levels of expression of the maize leaf SPS. The highest SPS activities were found in lines 3812–9 and 3812–11 and activities similar to the control were found in lines 3812–14 and 3812–18. Figure 1 shows typical results obtained for the control, 3812–2, and 3812–11 populations. The transgenic tomato plants were divided into low, medium, and high expressors of SPS activity (Table I). The tomato plants appeared to tolerate the elevated levels of SPS with no apparent detrimental effects. The transformed plants that had extractable SPS activities virtually identical to the control plants represent useful additional internal controls because they were obtained by the same transformation process as those expressing high SPS activities and yet lack the maize SPS enzyme (Worrell et al., 1991). In the following discussion, only data from untransformed controls and transformed plants expressing the maize leaf SPS cDNA construct under the control of the promoter from a gene

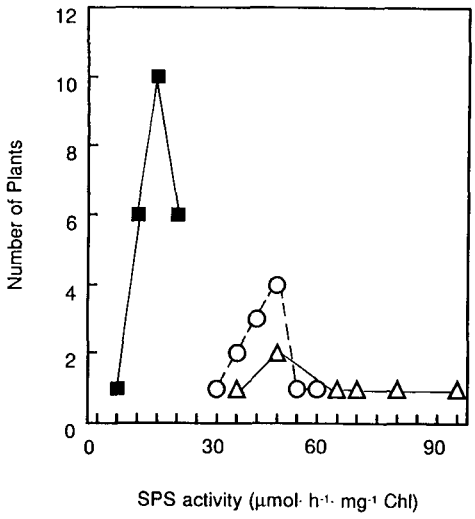

Figure 1. The range of extractable leaf SPS activities in the populations of control (UC82B, ■) and two transformed lines (3812–2, ○, and 3812–11, △) of tomato.

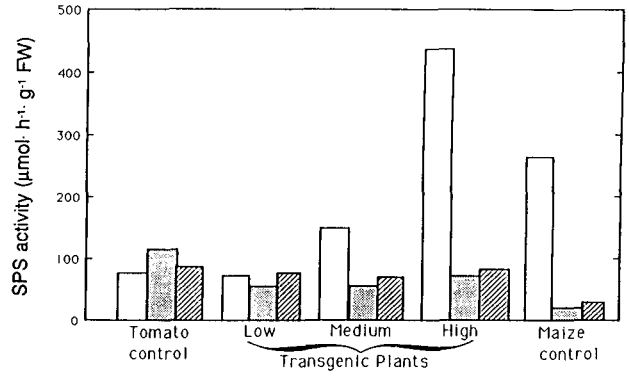

Figure 2. The effect of antibody to maize SPS on extractable SPS activity. Desalted leaf extracts were prepared from tomato and maize control plants, and from low-, medium-, and high-expressor transgenic plants. The extracts were incubated in either the absence of antibody (□) or with 0.5 (□) or 1.0 μL (▨) of purified monoclonal antibody specific for maize SPS. After 30 min on ice, a small volume of washed Immunoprecipitin was added, and after a further 15 min, the mixture was centrifuged. SPS activity in the supernatant was assayed under V_{max} conditions.

encoding the light-regulated and leaf-specific ribulose-1,5-bisphosphate carboxylase small subunit of tobacco (Worrell et al., 1991) will be considered.

It was thought possible that high expression of the maize SPS gene could reduce expression of the native tomato SPS gene(s). This question was tractable because monoclonal antibodies that recognize (and can immunoprecipitate) the native maize SPS molecule but do not cross-react with the tomato enzyme were available (Bruneau et al., 1991). Consequently, leaf extracts were prepared from several transgenic plants and the extracts were pretreated with the maize SPS-specific monoclonal antibodies followed by a precipitating agent (Immunoprecipitin, Gibco-BRL) to remove the immune complexes that formed. Subsequently, the V_{max} activity of the SPS remaining in solution was measured. SPS activity in extracts prepared from the tomato control plants was unaffected by preincubation with the maize SPS-specific monoclonal antibodies (Fig. 2), whereas SPS activity was almost completely removed from an extract prepared from maize leaves. Extracts were also prepared from low-, medium-, and high-expressing transformants. Treatment of these extracts with excess amounts of the maize-specific monoclonal antibodies reduced the activity in the extracts to roughly that found in the untransformed controls, i.e. about 70 μmol g^{-1} h^{-1}.

Table I. *SPS activities of leaves from untransformed and transformed tomato plants*

Leaves were harvested at midday in the light or following 16 h of darkness and assayed for extractable SPS activity. Values are means ± SE from at least three determinations.

Plant Type	SPS Activity		Increase upon Illumination
	Dark	Light	
	μmol h^{-1} mg^{-1} Chl		%
Untransformed			
UC82B	6.0 ± 2.2	11.3 ± 2.7	88
Transformants			
Low (3812-18)	7.6 ± 2.4	15.4 ± 4.2	103
Medium (3812-2)	34.0 ± 16.0	41.0 ± 9.0	21
High (3812-9)	48.0 ± 18.0	63.0 ± 16	31

Effects of Light-Dark Transitions on SPS Activity

In untransformed tomato plants, the extractable SPS activity obtained from leaves harvested at midday in the light was approximately double that measured at the same time in leaves from plants that had been maintained in 16 h of constant darkness (Table I). Thus, the native SPS activity of the tomato leaf shows significant inhibition in darkness, indicating that diurnal regulation of the enzyme occurs. In a transformant (3812–18) showing low expression of SPS (Fig. 1), the relative increase in enzyme activity upon illumination was double that in the untransformed controls. However, lines showing medium and high levels of expression of SPS (3812–2 and –9) had very high levels of enzyme activity in darkness, and the relative increase following illumination was much reduced (Table I). In these transformants, the increase in SPS activity in the light was only slightly more than that accounted for by activation of the native forms of the enzyme alone. It appears that when the maize leaf SPS is expressed in tomato, the enzyme is not regulated, and this results in high levels of SPS activity in both the light and the dark.

A Comparison of Photosynthesis in Air and Light CO_2

No differences in the CO_2 assimilation rate between the controls and transgenics were observed at low irradiances (<400 μmol m^{-2} s^{-1}) when photosynthesis was measured in air (Fig. 3) in Versailles or Raleigh. At high irradiances, the

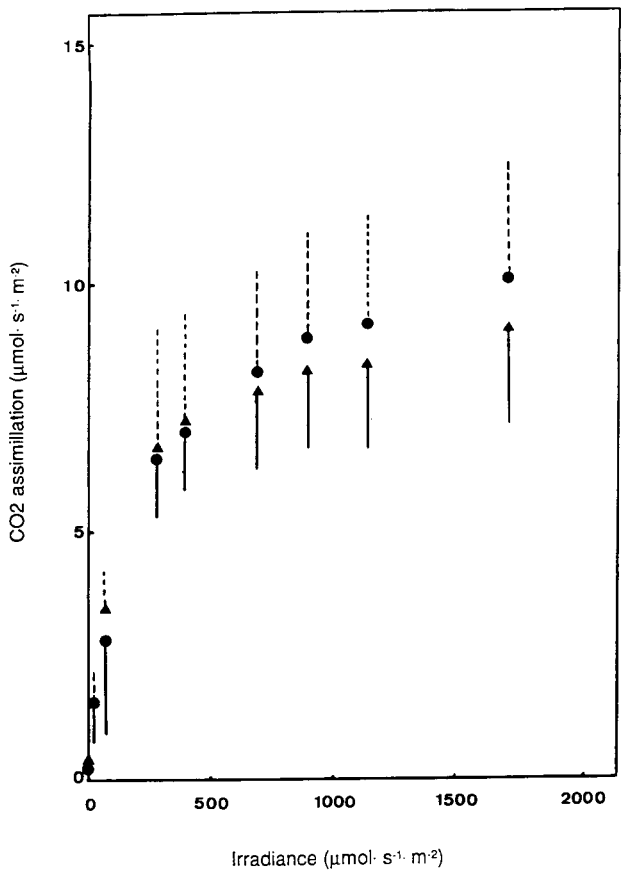

Figure 3. The light response curves for photosynthesis in air of high SPS-expressing transgenic tomato leaves (3812–9, ●) and untransformed controls (▲).

rate of CO_2 assimilation was marginally higher in the high SPS expressors than in the low SPS expressors and the controls (Fig. 3). However, the variation between leaf samples was large and the differences were not statistically significant. In contrast, when photosynthesis was measured in saturating CO_2, the difference between the controls and high SPS expressors was statistically significant at high irradiances (Fig. 4). In saturating CO_2 and light there is an increase in maximum photosynthetic capacity of about 15 to 20% in the high expressors relative to the controls (Table II). The difference in photosynthetic capacity between high and low SPS lines was most apparent at high light and high temperature (25°C versus 15°C). The activation state and total activity of ribulose-1,5-bisphosphate carboxylase was similar in all plants under equivalent conditions. This was also true of nitrate reductase and phospho*enol*pyruvate carboxylase activities (data not shown).

The Induction Phase of Photosynthesis

We compared the length of the induction period of photosynthesis in control and transgenic tomato plants and found no differences. High light to low light transitions in which leaves were allowed to photosynthesize in saturating light and CO_2 and then were subjected to brief interruptions in

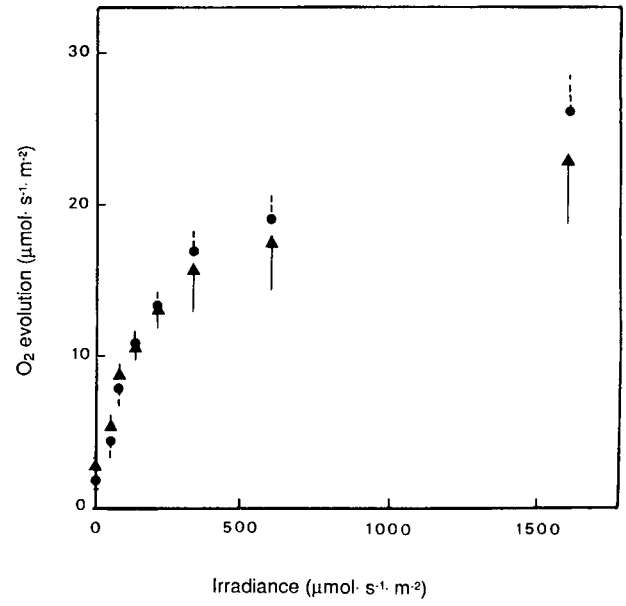

Figure 4. The light response curves for photosynthesis in saturating CO_2, measured in the leaf disc oxygen electrode, of transgenic (3812–9, ●) and untransformed control (▲) tomato plants.

low light followed by a return to high light were studied. Upon returning to high light there was a characteristic temporary enhancement of photosynthesis (Stitt, 1986) that was maximal following a 30- to 60-s exposure to low light. In the untransformed plants, the temporary enhanced rate of photosynthesis was about 50% above the steady-state rate of O_2 evolution (Fig. 5), but in the transgenic tomatoes expressing high SPS activities, the elevated rate could be up to 200% above the steady-state rate of CO_2 assimilation (Fig. 5).

Metabolite Levels

A comparison of the metabolite levels in transformed and untransformed plants showed that differences in the leaf Glc6P, Fru6P, and UDPGlc levels (Table III) were small. The Glc6P to Fru6P ratio decreased with increasing SPS activity. This result, in conjunction with a slight decrease (about 15%) in UDPGlc, is consistent with increased activity of SPS in the transgenic plants.

Table II. *High expression of the maize SPS gene in transgenic tomato plants increases maximum photosynthetic capacity*

Values are means ± SE from at least three determinations.

Expression Level	SPS	Maximum Photosynthetic Capacity
	μmol h^{-1} g^{-1} fresh weight	*μmol O_2 s^{-1} m^{-2}*
Low	98	24.7 ± 1.1
Medium	189	26.6 ± 1.7
High	462	28.3 ± 1.8

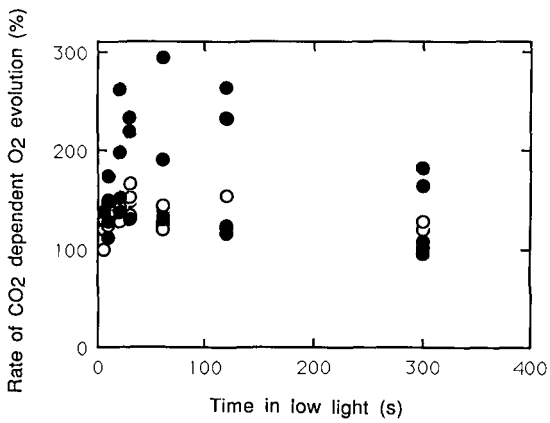

Figure 5. The enhancement of photosynthesis following exposure to low light conditions (40 μmol s^{-1} m^{-2}). Leaf discs were illuminated with saturating light (1600 μmol s^{-1} m^{-2}) until steady-state rates of photosynthesis were obtained. They were then exposed to low light for periods of varying duration before they were returned to high light. Photosynthetic O_2 evolution measured in transgenic (●) and untransformed (○) tomato plants is expressed as a percentage of the steady-state rate.

Effects on Starch, Suc, and Hexose Sugar Contents of Leaves

The ratio of starch to Suc in leaves provides an indicator of carbon partitioning. Transgenic plants expressing high activities of maize SPS accumulated more Suc, Glc, and Fru and less starch compared with low expressors and wild-type plants (Fig. 6). There was a strong positive correlation between the leaf Suc:starch ratio and the leaf SPS activity in plants grown in a growth chamber in Raleigh (Fig. 6A) and in the glasshouse in Versailles (Fig. 6B).

Effects on Plant Growth

The overall growth of vegetative plants was not increased with increased expression of SPS. However, the partitioning of dry matter within the plant was altered in the transgenic plants (Fig. 7). As leaf SPS activity was increased, the proportion of the total dry matter in leaves tended to increase,

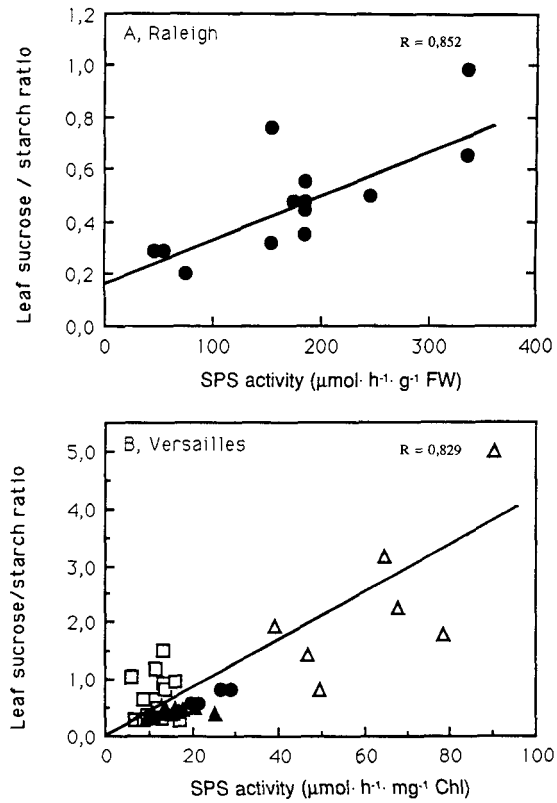

Figure 6. The relationship between the leaf Suc:starch ratio and SPS activity in mature leaves of control and transgenic tomato plants. Leaves were harvested after 8 h of illumination from a growth chamber in Raleigh (A). In Versailles, plant material harvested at midday from the glasshouse (B) consisted of controls (□) and 3812-18 (▲), 3812-2 (●), and 3812-9 (△) transformants.

whereas that in the roots tended to decrease (Fig. 7). The proportion allocated to the stem fraction remained relatively constant (Fig. 7). The changes in the percentage increase in leaf and stem dry weights were not statistically significant, whereas the negative correlation between relative root dry weight and SPS activity was statistically significant at the 0.01 level.

The shoot:root dry weight ratio was positively correlated with leaf SPS activity (Fig. 8). Differences in shoot:root ratio between low and high expressors approached 2-fold. These results clearly demonstrate that carbon metabolism can influence plant partitioning of resources.

Table III. *Metabolite levels in transgenic tomato plants expressing the maize SPS gene in two different experiments*

For relative SPS activities, see Table IV and Figure 1.

SPS Expression Level	Metabolite Level				Glc6P:Fru6P Ratio
	Glc6P	Fru6P	Glc1P[a]	UDPGlc	
	µmol g^{-1} fresh weight				
Low	260	46	37	100	5.7
	169	44		92	3.8
Medium	110	27	20	90	4.1
	169	43		66	3.9
High	140	42	24	84	3.3
	148	45		81	3.3

[a] Glucose 1-phosphate.

Enhanced Expression of SPS in Other Plant Parts

Although the maize SPS gene was targeted to the cytosol of leaf mesophyll cells (Worrell et al., 1991), it is possible that some expression of the maize gene also occurred in other plant parts, especially Chl-containing tissues such as the petiole. As shown in Table IV, SPS activity was elevated 3- to 4-fold in leaves, petioles, and roots of high expressors relative to low expressors. Although absolute SPS activities were highest in leaves, substantial activities were also found in other plant parts.

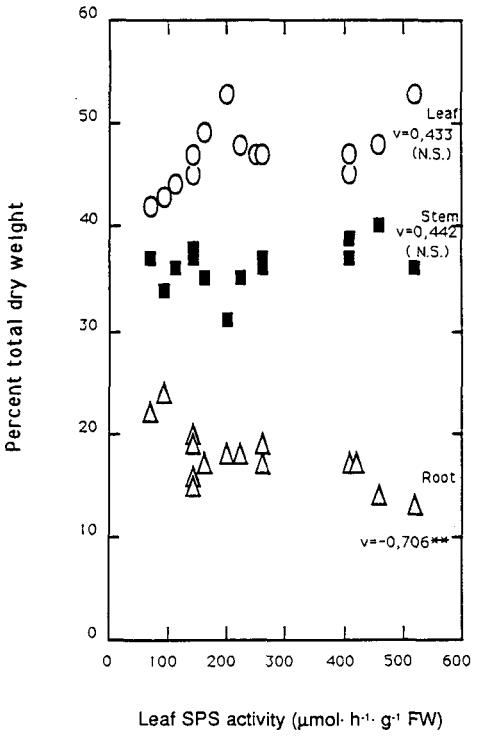

Figure 7. Alteration in dry matter partitioning between untransformed and transgenic tomato plants. Leaf (○), stem (■), root (△).

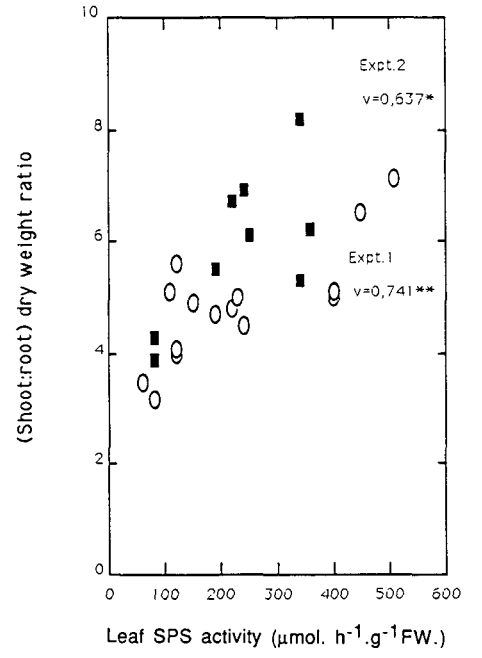

Figure 8. The relationship between the shoot:root dry weight ratio and SPS (V_{max}) activity in mature leaves of untransformed and transgenic plants. Results from two separate experiments are shown.

Table IV. *SPS activity and Suc in different parts of transgenic tomato plants*

Tissue	SPS		Suc	
	Low expressor	High expressor	Low expressor	High expressor
	µmol h^{-1} g^{-1} fresh weight		*µmol g^{-1} fresh weight*	
Leaf	89	385	13	21
Petiole	12	42	ND[a]	ND
Root	10	33	4.7	9

[a] Not determined.

There was an overall positive relationship between SPS activity in leaves compared with roots (Fig. 9). The shoot:root dry weight ratios tended to be higher in experiment 2 (Fig. 8) than in experiment 1 (Fig. 9), which may reflect the fact that the plants were slightly younger at harvest. It appears that the maize SPS gene is expressed to about the same relative extent in all plant tissues (i.e. high expressors have absolute SPS activity increased 3- to 4-fold).

The root sucrose content was generally elevated in the transgenic plants (Fig. 10, Table IV). It is possible that the steady-state concentration of Suc in roots of transgenic plants is increased because of enhanced Suc synthesis (catalyzed by SPS), and this occurs simultaneously with Suc degradation. Assuming that the Suc pool represents the balance between synthesis and degradation, an increased rate of Suc synthesis in the transgenic plants could account for the increase in the Suc pool. There was a strong negative correlation between root SPS activity and relative root growth (proportion of total dry matter partitioned to the root, Fig. 9).

DISCUSSION

The regulation of Suc synthesis provides a means of altering the partitioning of photosynthate between starch and Suc and also modulating the synthesis and export of photosynthate via the release and recycling of Pi. An increase in the capacity for Suc synthesis might, therefore, be predicted to incur changes in assimilate partitioning in the leaf and, hence, assimilate export. Similarly, an increase in SPS activity might also be predicted to favor an increase in production at the

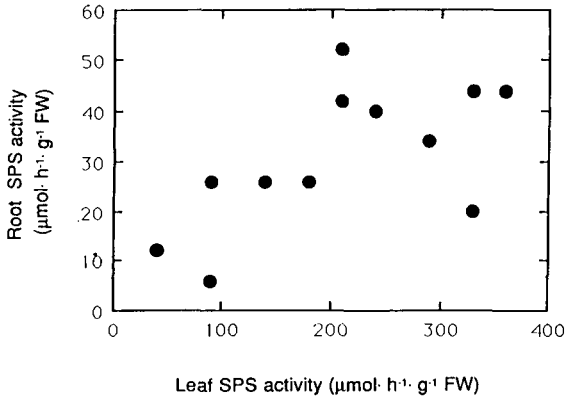

Figure 9. The relationship between leaf SPS and root SPS activities in transgenic tomato plants.

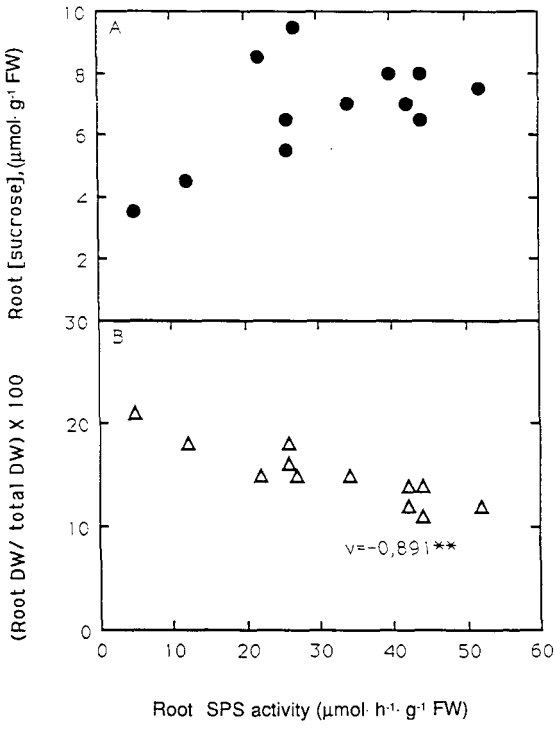

Figure 10. The relationship between root SPS activity and the root Suc content (A) and the relative root dry matter content (B) in transgenic tomato plants.

source and stimulate photosynthesis. In the data presented here, we show that all these effects can be observed as a result of elevating the endogenous SPS activity. Furthermore, we have demonstrated that the partitioning of assimilate between Suc (and hexoses) and starch within the leaf is dictated by the level of SPS activity. The availability of assimilate is determined by its rate of production in the source (i.e. source strength) (Ho, 1978, 1979).

In tomato, there is a linear relationship between the rate of carbon transport and the rate of carbon assimilation. We have observed an increase in the steady-state rate of photosynthesis in conditions of saturating light and CO_2 and also a transient enhancement of photosynthetic rate following a short exposure to low light in the high SPS plants compared with the controls. Therefore, the rate of Suc export must also be enhanced under these conditions. In addition, the increase in leaf SPS results in a change in the partitioning of assimilate at the whole-plant level, which favors the incorporation of dry matter into the shoot as opposed to the root, suggesting that patterns of assimilate export are modified in response to increases in SPS activity.

The basis for the shift in whole plant dry matter partitioning is not clear. One contributing factor, for which there is some experimental support, is that root growth in the high SPS expressors is decreased. Decreased partitioning of dry matter to the root was correlated with increased root SPS activity and an increased root Suc level. This may cause an imbalance in the sugar metabolism of the root. Tomato is considered to be a high leaf-starch former (Ho, 1978; Hammond et al., 1984) with Suc and hexoses being the predominant soluble carbohydrates stored in the leaf (Ho, 1978, Ho, 1979; Hammond et al., 1984). The increase in leaf SPS activity completely inverses this tendency and the leaves of the high SPS expressors accumulate more Suc and hexoses and less starch. Remarkably, this has no apparent detrimental effects because photosynthesis and growth are comparable to or even higher than in the low SPS expressors and controls. In conditions of saturating light and CO_2, the rate of photosynthesis was increased by up to 20% in the high SPS expressors relative to controls. In air at nonsaturating irradiance, no significant differences in assimilation rate were measured, although changes in partitioning were evident. At nonsaturating irradiance the light is the limiting factor for photosynthesis.

The response of photosynthesis to brief exposures to low light might be considered to be analogous to the light-fleck response of leaves (Kirschbaum and Pearcy, 1988). The occurrence of a light-fleck in shade-adapted plants induces a burst of O_2 evolution as a result of increased noncyclic electron transport. The temporary enhancement in the rate of photosynthesis after a brief exposure to low light in high-light-adapted plants is associated with marked changes in metabolite levels similar to those occurring during the light-fleck response (Stitt, 1986; Kirschbaum and Pearcy, 1988). Carbon gain in these situations can be twice that predicted from steady-state photosynthesis.

The period of enhanced photosynthesis is short because the rate of electron transport is rapidly constrained by the processes of photosynthetic control (Stitt, 1986). It is interesting to note that the capacity for Suc synthesis plays a role in the overall capacity for enhancement of the photosynthetic rate following exposure to low light. This is evidenced by the greater enhancement in photosynthetic rate in the high SPS expressors than in control leaves (Fig. 5). It is interesting to speculate that the possession of elevated SPS activities might be advantageous in fluctuating light environments. However, the measurements in Figure 5 were made at saturating CO_2, which markedly alters metabolite levels and may be of no relevance to light use efficiency in air. These effects are only important when the supply of light and CO_2 are adequate and the capacity for Suc synthesis becomes the principal factor limiting photosynthesis.

The regulation of Suc formation in leaves has often been considered in terms of the metabolic control exerted by regulation of cytosolic Fru-1,6-bisphosphatase (Stitt et al., 1980; Huber, 1983; Stitt et al., 1983; Stitt and Heldt, 1985; Stitt et al., 1985, 1987; Kerr and Huber, 1987; Stitt and Quick, 1989). However, it has always been recognized that control of SPS was also important (Huber, 1983; Kerr and Huber, 1987; Stitt and Quick, 1989).

Control of SPS activity was evident from changes in the activity measured in crude extracts during the photoperiod and in response to sink manipulation (Kerr et al., 1985; Kerr and Huber, 1987; Stitt and Quick, 1989). Changes in the amount of extractable SPS activity in vivo in standard assay conditions were evident in tomato extracts produced from dark- and light-adapted leaves (Table I). This is in contrast to the observation of Worrell et al. (1991), who found that the reduction in SPS activity at night in tomato leaves was not pronounced.

In maize leaves, SPS is known to be subject to light/dark modulation regulated by protein phosphorylation (Kalt-Torres and Huber, 1987; Huber and Huber, 1991). However, it is clear from the results presented here and those of Worrell et al. (1991) that when maize SPS is expressed in tomato leaves its light/dark regulation (Huber et al., 1987) is lost, so that the maize enzyme has a high activity in both light and dark. This feature must be important in causing at least some of the metabolic and physiological effects observed in this study. In addition, it is clear from the metabolite measurements that when SPS activity is increased there is not a compensating restriction of the cytosolic Fru-1,6-bisphosphatase reaction to throttle back the flow of carbon to Suc. Thus, it appears that the regulation of SPS activity is more important in determining the rate of Suc synthesis than is regulation by Fru-1,6-bisphosphatase activity. Because direct product inhibition by Suc-6-P does not appear to play an important role in the regulation of SPS (Krause and Stitt, 1992), we conclude that the activity of this enzyme is the major factor in determining flux through the pathway of sucrose synthesis.

High expression of the maize SPS gene does not suppress tomato SPS gene expression (Fig. 2). The data in Figure 2 also suggest that heterologous SPS molecules composed of maize and tomato subunits probably do not occur. However, heterologous forms may be inactive or the anti-maize SPS antibodies might not be capable of recognizing and immunoprecipitating these molecules from solution, for example, if the monoclonal antibody recognized the interface region between homologous subunits. Estimates of the native M_r of SPS vary from about 250,000 to 450,000 (Doehlert and Huber, 1983; Salvucci et al., 1990). Because the subunit M_r of the maize enzyme is about 130,000 (Walker and Huber, 1989; Bruneau et al., 1991), the active form of the native enzyme is either a dimer or a tetramer. There is no evidence in the literature that suggests that the monomeric subunit possesses enzymic activity. Thus, it is intriguing to ask why heterologous or chimeric molecules do not occur in vivo. Perhaps only the interactions between homologous subunits are stable.

A role for sugars such as Suc in source-sink coregulation and also in gene regulation has been proposed. It remains to be demonstrated whether the changes in the tomato leaf carbohydrate status resulting from increases in SPS activity have implications for these processes. However, as shown by the data presented here, the increase in leaf SPS is wholly beneficial, with no detrimental effects on source-sink relationships or gene expression being immediately apparent.

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