Planta (1995) 196:327–334

Planta

Altered photosynthesis, flowering, and fruiting in transgenic tomato plants that have an increased capacity for sucrose synthesis

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Received: 13 April 1994 / Accepted: 9 August 1994

Abstract. Photosynthesis, leaf assimilate partitioning, flowering, and fruiting were examined in two lines of *Lycopersicon esculentum* Mill. transformed with a gene coding for sucrose-phosphate synthase (SPS) (EC 2.3.1.14) from *Zea mays* L. expressed from a tobacco ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) small subunit promoter. Plants were grown at either 35 or 65 Pa CO_2 and high light (1000 μmol photons·m^{-2}·s^{-1}). Limiting and maximum SPS activities were significantly greater (up to 12 times) in the leaves of SPS-transformed lines for all treatments. Partitioning of carbon into sucrose increased 50% for the SPS transformants. Intact leaves of the control lines exhibited CO_2-insensitivity of photosynthesis at high CO_2 levels, whereas the SPS transformants did not exhibit CO_2-insensitivity. The O_2-sensitivity of photosynthesis was also greater for the SPS-transformed lines compared to the untransformed control when measured at 65 Pa CO_2. These data indicate that the SPS transformants had a reduced limitation on photosynthesis imposed by end-product synthesis. Growth at 65 Pa CO_2 resulted in reduced photosynthetic capacity for control lines but not for SPS-transformed lines. When grown at 65 Pa CO_2, SPS transformed lines had a 20% greater photosynthetic rate than controls when measured at 65 Pa CO_2 and a 35% greater rate when measured at 105 Pa CO_2. Photosynthetic rates were not different between lines when grown at 35 Pa CO_2. The time to 50% blossoming was reduced and the total number of inflorescences was significantly greater for the SPS transformants when grown at either 35 or 65 Pa CO_2. At 35 Pa CO_2, the total fruit number of the SPS transformants was up to 1.5 times that of the controls, the fruit matured earlier, and there was up to a 32% increase in total fruit dry weight. Fruit yield was not significantly different between the lines when grown at 65 Pa CO_2. Therefore, there was not a strict relationship between yield and leaf photosynthesis rate. Flowering and fruit development of the SPS-transformed lines grown at 35 Pa CO_2 showed similar trends to the controls grown at 65 Pa CO_2. Incidences of blossom-end rot were also reduced in the SPS-transformed lines. These data indicate that altering starch/sucrose partitioning by increasing the capacity for sucrose synthesis can affect acclimation to elevated CO_2 partial pressure and flowering and fruiting in tomato.

Key words: Carbon partitioning – Carbon dioxide acclimation – *Lycopersicon* – Photosynthesis – Sucrose and starch synthesis – Sucrose-phosphate synthase

Introduction

Sucrose is one of the primary end products of photosynthesis in higher plants. It is also the major carbohydrate transported to sink tissues for plant growth and development (Pate 1976). Since sucrose is the product of photosynthesis "seen" by the rest of the plant, it is the interface between photosynthesis and plant growth and development.

Gas-exchange experiments have shown that the capacity for end-product synthesis (triose-phosphate utilization) can limit the rate of photosynthesis (Sharkey and Badger 1984; Leegood 1989; Sharkey 1990). An insensitivity of photosynthesis to O_2 and CO_2 (or reversed sensitivity) is diagnostic of an end-product limitation on photosynthesis (Sharkey 1990). This limitation on photosynthesis occurs under conditions favoring high rates of triose-phosphate production by the chloroplast, such as high light and high CO_2 levels (Jolliffe and Tregunna

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Abbreviations: DAS=days after seeding; *npt*II=neomycin phosphotransferase; Rubisco=ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP=ribulose-1,5-bisphosphate; SPS=sucrose-phosphate synthase; SSU=Rubisco small subunit

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1968; Sharkey and Badger 1984; Sharkey 1990). Harley et al. (1992) and Socias et al. (1993) have demonstrated that end-product synthesis can limit photosynthesis when plants are grown at high partial pressures of CO_2. It is predicted that atmospheric levels of CO_2 will nearly double to 65 Pa in the next 60 years (Bowes 1993), and so end-product limitations on photosynthesis will become more significant in the future.

Plants that have genetically determined alterations in their sucrose synthesis capacity provide an opportunity to examine the interactions between sucrose synthesis and photosynthesis and plant growth. Worrell et al. (1991) genetically manipulated the sucrose synthesis pathway in tomato by transforming plants with a cDNA clone coding for sucrose-phosphate synthase (SPS) from maize. The maize SPS was placed under the control of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) small subunit promoter (SSU promoter) from tobacco to allow preferential expression in leaf tissue. The maximum activity of SPS was significantly increased in the leaves of tomato plants expressing the maize SPS, and the absolute levels of starch and sucrose in the leaves were altered in the predicted manner (Worrell et al. 1991).

Galtier et al. (1993) examined the photosynthetic characteristics of these SPS transformants when grown at present atmospheric levels of CO_2 (35 Pa). No significant differences were found in response to changing levels of light and CO_2, although at saturating light and CO_2 there was some indication that the transgenic plants had a higher rate of photosynthesis. However, the SPS transformants were not grown under conditions favoring O_2- and CO_2-insensitive photosynthesis (e.g. high light and high CO_2 levels). It can be predicted that an increased capacity for sucrose synthesis will have greater effects on photosynthesis when plants are grown at high CO_2 levels.

The SPS transformants also provide the opportunity to study interactions between plant development and sucrose synthesis. Galtier et al. (1993) examined the early vegetative growth of the SPS-transformed lines and found that the shoot to root ratio was greatest in the transformants during early vegetative growth, indicating a shift in whole-plant carbon partitioning. Lejeune et al. (1993) have speculated that sucrose levels can influence flowering in some plant species. Friend et al. (1984) showed that flowering of *Brassica campestris* in culture is stimulated by increased levels of sucrose in the media. Therefore, we wanted to study flowering and fruit development in the SPS transformants.

In this study, two SPS-transformed lines (SSU-9 and SSU-11), one kanamycin-resistant control line (TCon-1), and one untransformed control line (UC82B) were grown at either 35 or 65 Pa CO_2 and high light (1000 μmol photons·m^{-2}·s^{-1}). Leaf SPS activities were determined at two stages of development, and leaves were labeled with $^{14}CO_2$ to examine leaf assimilate partitioning. Leaf photosynthesis was measured over a range of CO_2 levels (5–105 Pa), and O_2-sensitivity of photosynthesis was measured at 35 and 65 Pa CO_2. Vegetative growth, flowering, and fruiting were also followed throughout development.

Materials and methods

Plant material and growth conditions. Tomato (*Lycopersicon esculentum* Mill.) cultivar UC82B was transformed using *Agrobacterium tumefaciens*. The generation of lines SSU-9 and SSU-11 from pCGN3812 has been previously described (Worrell et al. 1991). Briefly, pCGN3812 contains the construct 5'-35S-*npt*II-*tml*-3'/5'-tobacco Rubisco SSU-maize SPS cDNA-3'. pCGN1547 (5'-*mas*-*npt*II-*mas*-3') has been previously described (McBride and Summerfelt 1990). Co-cultivation of UC82B with pCGN1547 to yield TCon-1 was performed as described by Fillatti et al. (1987). Worrell et al. (1991) confirmed that lines SSU-9 and SSU-11 expressed the maize SPS by Western blots. The T2 generation was used for all studies except the $^{14}CO_2$-feeding experiments where T3 plants were used.

Plant material was grown either in growth chambers at the University of Wisconsin Biotron (for the $^{14}CO_2$-feeding experiment) or in Conviron E-15 growth chambers (for all other experiments). In the Biotron, plants were illuminated by metal-halide lamps at a peak level of 500 μmol photons·m^{-2}·s^{-1} (pot level), 26°C for the 16-h day and 18°C at night, and a relative humidity of 60%. Plants were watered daily with half-strength Hoagland's solution (Hoagland and Arnon 1938) using an automatic drip-irrigation system. In the Conviron E-15 growth chambers, plants were illuminated by metal-halide+incandescent lamps at a peak level of 800 (pot level) to 1200 (top of mature plants) μmol photons·m^{-2}·s^{-1}, 35 or 65 Pa CO_2 (for the entire 24-h period), 26°C for the 16-h day and 18°C at night, and a relative humidity of 60%. Plants were watered manually with half-strength Hoagland's solution every day. A gradient of 1.5 h for both light and temperature was used during the day/night transitions in all growth chambers. Plants were grown in 6 L pots using a soil-less potting mix (Metromix 360; W.R. Grace and Co., Cambridge, Mass., USA). During flowering, plants were shaken manually once each day to aid in pollination.

In experiments where the untransformed control UC82B was compared to the SPS-transformed lines SSU-9 and SSU-11, seeds were germinated in flats using Metromix 360 and then transplanted into 6 L pots after 7 d. High-SPS expressors were selected from lines SSU-9 and SSU-11 by measuring SPS activities, and not on the basis of kanamycin resistance. Selection for kanamycin resistance was not used in these experiments since preliminary experiments showed that the kanamycin selection procedure significantly stunted the early growth of lines SSU-9, SSU-11 and TCon-1 (up to 7 d) compared to controls where kanamycin was not included in the selection media. In experiments where the kanamycin-resistant control TCon-1 was compared to the SPS-transformed lines, a kanamycin selection was performed. Kanamycin-resistant plants were selected by culturing seeds in a liquid media containing 100 mg·L^{-1} kanamycin sulphate for 10 d; resistance to kanamycin was determined by the ability of roots to grow in kanamycin media. High-SPS expressors were then selected from lines SSU-9 and SSU-11 by measuring SPS activities.

Gas-exchange measurements and $^{14}CO_2$- labeling procedures. Intact leaves used for gas-exchange measurements were randomly selected from the plants, although very young and very old leaves were avoided. Measurements were made between 35–43 d after seeding (DAS), and plants were randomly selected throughout the day to control for diurnal effects. One intact leaflet from each compound leaf was clamped into an aluminum cuvette with a glass window on the top surface. Modeling clay was used to seal the petiole into the cuvette. Leaf temperature was controlled using Peltier blocks; with this device the leaf temperature was maintained within a tolerance of ±0.2°C. A copper-constantan thermocouple was pressed along the abaxial surface of the leaf to measure leaf temperature; leaf temperatures were maintained at 26°C. Air within the chamber was mixed using two small fans to reduce the boundary layer. White light was provided by a 2.5-kW, air-cooled, xenon-arc lamp. Infrared light was removed using a combination of cold and hot mirrors and infrared-absorbing glass cooled with

flowing water. Light levels were measured using a quantum sensor (model LI-189; Licor, Lincoln, Neb., USA). Air entering the cuvette was mixed from N_2, O_2, and 5% CO_2 in air using Datametrics type 825 mass-flow controllers. This allowed the partial pressures of CO_2 and O_2 to be controlled in the cuvette; the partial pressures of these gases used for photosynthetic measurements are indicated in the figure legends. The partial pressure of CO_2 before and after passage through the cuvette was measured using a Licor 6262 CO_2/H_2O analyzer. Either dry or humidified air was passed into the cuvette. When humidified air was used, the humidity was controlled by passing CO_2-free air through water followed by condensation in a copper coil held in a water bath at the chosen dewpoint. Humidity was measured before and after the cuvette using the Licor 6262 CO_2/H_2O analyzer. The measurement conditions used for each experiment are described in the figure legends. Calculations of gas-exchange parameters were done as described by von Caemmerer and Farquhar (1981). O_2-sensitivites were determined using the following equation: $[P_N$ (2 kPa O_2) $-P_N$ (20 kPa O_2)$]/P_N$ (2 kPa O_2), where P_N=photosynthesis. Theoretical O_2-sensitivities were calculated as described by Sage and Sharkey (1987) using the kinetic constants given by Jordan and Ogren (1984). Leaf areas were determined by tracing the pattern of leaves on paper, cutting the traces out, and then calculating the leaf area by weighing the paper.

In the ^{14}C-labeling experiment, intact tomato leaves were placed into an aluminum cuvette as described above. Leaves were left to equilibrate at 500 µmol photons·m^{-2}·s^{-1}, 26°C, 35 Pa CO_2, and 20 kPa O_2 (growth conditions) for at least 30 min using the gas-exchange system described above. Leaves were labeled by switching the 5% CO_2 supply from $^{12}CO_2$ in air to a tank of $^{14}CO_2$ in air (specific activity 0.9 MBq·mol^{-1}). The $^{14}CO_2$ feedings were performed under steady-state conditions at the conditions stated above. A 5-min pulse of $^{14}CO_2$ and a 10-min chase period were used. A 10-min chase period was chosen since earlier work showed that most label is out of the photosynthetic carbon-reduction cycle at this time, but label has not been exported from the leaf (Sharkey et al. 1985). The rate of photosynthesis was measured just prior to the feeding and again after the feeding during the chase period. If these measurements differed by 10%, the leaves were discarded as not being at steady state. After the chase, leaves were cut at the petiole and removed from the cuvette, wrapped in aluminum foil, and frozen in liquid N_2. Leaves were stored at −80°C until analyzed. Additional details are given in Sharkey et al. (1992).

Biochemical determinations. The $^{14}CO_2$-labeled leaf material was extracted, and the radioactivity in the starch, neutral (primarily water-soluble carbohydrates), and ionic (cationic+anionic) fractions quantified as previously described (Sharkey et al. 1992).

For the analysis of leaf SPS activity, a cork borer (0.5 cm^2 in diameter) was used to randomly sample 20 leaves per plant in the middle of the light period, the tissue was placed in aluminum foil, and then immediately frozen in liquid N_2. The tissue was stored at −80°C until analyzed (within two weeks of sampling). Leaf extracts were prepared and assayed as described by Huber et al. (1991) with minor modifications.

Leaf tissue used for Rubisco assays was sampled as described above for the SPS assay. Leaf tissue was extracted, and then analyzed for initial and total Rubisco activities, and Rubisco protein levels, as described by Sharkey et al. (1991).

Plant harvest and dry-weight determinations. Root tissue was separated from the growth media by washing the roots with a running stream of water and soaking the roots in water several times. Metal screening was used to maximize the recovery of root material. Roots and shoots were dried for 4 d at 70°C in a forced air-drier. Tomato fruit were dried under the same conditions for 10 d.

Statistical analysis. Significance of differences between means was tested using the Student's *t*-test. A 5% probability level was considered significant.

Results

Activities of SPS and leaf assimilate partitioning. Leaf SPS activities, assayed using either limiting or saturating substrate concentrations, were substantially greater for the two SPS-transformed lines compared to line UC82B for all treatments (up to 12-fold greater) (Fig. 1A, B). At 40 DAS the SPS activities for plants grown at 65 Pa CO_2 were not different from those grown at 35 Pa CO_2 for all lines (Fig. 1A). However, in a different crop at 50 DAS the SPS-transformed lines grown at 65 Pa CO_2 showed a decrease in SPS activity compared to the SPS transformants grown at 35 Pa CO_2 (Fig. 1B). The SPS activities for line UC82B at 50 DAS were not significantly different between plants grown at either 35 or 65 Pa CO_2. The transformed control line (TCon-1) had SPS activities similar to line UC82B for all treatments (data not shown).

Plants of the SPS-transformed lines partitioned much less photosynthate to starch and much more to water-soluble carbohydrates (sucrose) than did the control line (Table 1). The ^{14}C-ratio of starch to water-soluble carbohydrates was 0.7 in the control line but only 0.3 in the SPS-transformed lines.

Photosynthetic measurements. The photosynthetic rates of lines UC82B, SSU-9, SSU-11, and TCon-1 when grown at 35 Pa CO_2 were not significantly different for all CO_2 levels tested (Fig. 2A). Line UC82B and the SPS transformants showed a maximum photosynthetic rate of

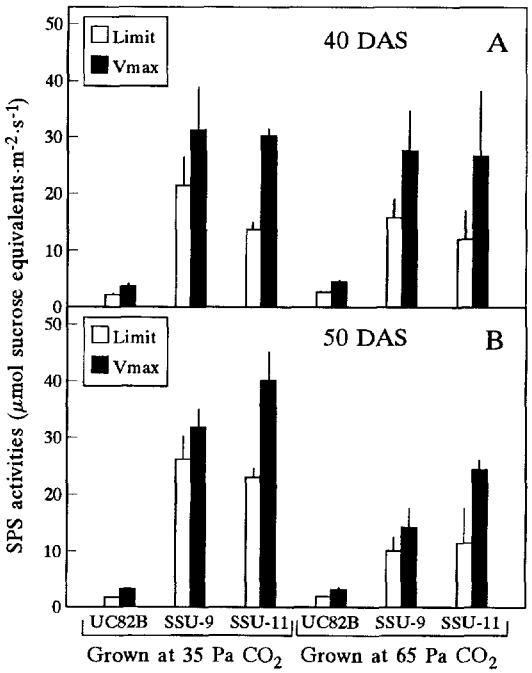

Fig. 1A, B. Leaf SPS activities for untransformed (line *UC82B*) and SPS transformed tomatoes (lines *SSU-9* and *SSU-11*) at 40 (**A**) and 50 (**B**) DAS. Plants were grown at 35 or 65 Pa CO_2 and 1000 µmol photons·m^{-2}·s^{-1}. The assays were performed using limiting substrate concentrations (plus inorganic phosphate) (*Limit*) or saturating concentrations of substrates (V_{max}) as determined for spinach (Huber et al. 1991). Tissue was sampled in the middle of the light period. Values represent the mean±SE; n=3 plants per treatment

Table 1. The proportion of total leaf ^{14}C in starch, water-soluble carbohydrates (WSC), and the ionic fraction, and the starch/WSC ^{14}C-ratio, following a 5-min pulse of $^{14}CO_2$ and a 10-min chase for untransformed (UC82B) and SPS-transformed (SSU-9, SSU-11) tomato lines. The total ^{14}C (starch+WSC+ionic fraction) incorporated into the leaves was similar for each tomato line. Values represent the mean±SE; n=4–5 plants per treatment

Line	% of total ^{14}C			Starch/WSC
	Starch	WSC	Ionic	
UC82B	27±2	41±2	32±2	0.7±0.1
SSU-9	18±2	62±3	21±2	0.3±0.1
SSU-11	16±5	60±5	24±3	0.3±0.1

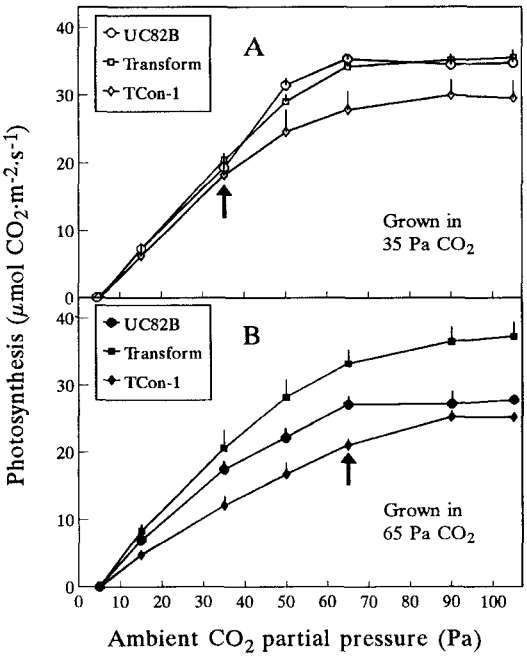

Fig. 2A, B. The responses of photosynthesis of intact leaves to changing partial pressures of CO_2 for different lines of tomato grown at 35 (**A**) or 65 (**B**) Pa CO_2 and 1000 μmol photons·m^{-2}·s^{-1}. The tomato lines included an untransformed control (*UC82B*), a kanamycin-resistant control (*TCon-1*), and two SPS transformants (*SSU-9* and *SSU-11*). The measurements for lines SSU-9 and SSU-11 were combined since no significant differences were found between them. The *arrows* indicate the partial pressure of CO_2 used for growth. Photosynthetic measurements were made at 1400 μmol photons·m^{-2}·s^{-1} (saturating light) and the indicated partial pressure of CO_2. Plants at 35–43 DAS were used. Values represent the mean±SE; n=6 plants from 2 separate experiments for lines UC82B, SSU-9, and SSU-11 or 3 plants from 1 experiment for line TCon-1

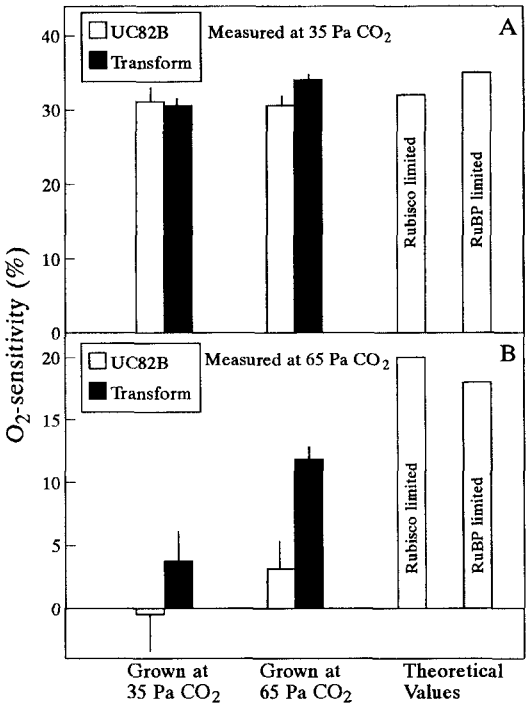

Fig. 3A, B. The O_2-sensitivity of photosynthesis determined at 35 (**A**) or 65 (**B**) Pa CO_2 for intact leaves of untransformed (*UC82B*) and SPS-transformed (*Transform*; SSU-9 and SSU-11) lines of tomato. Plants were grown at 35 or 65 Pa CO_2 and 1000 μmol photons·m^{-2}·s^{-1}. Measurements were made at saturating light (1400 μmol photons·m^{-2}·s^{-1}). Measurements for lines SSU-9 and SSU-11 were combined since no significant differences were found between them. The theoretically predicted O_2-sensitivities when photosynthesis is limited by either Rubisco or RuBP regeneration are also shown; partial pressures of CO_2 of 22 Pa (for measurements at 35 Pa CO_2) and 50 Pa (for measurements at 65 Pa CO_2) were used in the calculations as described by Sage and Sharkey (1987) using the kinetic constants of Jordan and Ogren (1984). Values represent the mean±SE; n=6 plants from 2 separate experiments

35 μmol CO_2·m^{-2}·s^{-1}, and line TCon-1 reached a maximum rate of 30 μmol CO_2·m^{-2}·s^{-1}. The photosynthetic rates at 35 Pa CO_2 (growth level) for the different lines were not significantly different and ranged from 18–20 μmol CO_2·m^{-2}·s^{-1}.

The SPS-transformed plants grown in 65 Pa CO_2 had higher rates of photosynthesis than controls regardless of the CO_2 partial pressure during the assay (Fig. 2B). Line TCon-1 (transformed control) had lower rates than line UC82B (untransformed control). The rates of photosynthesis at the growth level of CO_2 (65 Pa) were 33 (SPS-transformed lines), 27 (line UC82B), and 21 μmol CO_2·m^{-2}·s^{-1} (line TCon-1); the rate of photosynthesis of the SPS-transformed lines was significantly greater than that of the controls. Photosynthesis in line UC82B was insensitive to changes in CO_2 levels above 65 Pa and in TCon-1 it was insensitive above 85 Pa. The SPS transformants did not exhibit CO_2-insensitivity up to 105 Pa CO_2. The above relationships were also found when photosynthesis was plotted against intercellular CO_2 partial pressure (data not shown).

The sensitivity to CO_2 was difficult to judge accurately so sensitivity to O_2 was also determined. When measured at 35 Pa CO_2, all plants exhibited O_2-sensitivity in the range predicted when limited by either Rubisco or ribulose1,5-bisphosphate (RuBP) regeneration (Fig. 3A). The O_2-sensitivity of the SPS transformants was always greater than the control when measured at 65 Pa CO_2 (Fig. 3B), and O_2-sensitivity was always greater in plants grown at 65 Pa CO_2 (Fig. 3A). The O_2-sensitivity of the SPS transformants grown at 65 Pa CO_2 approached the theoretically predicted O_2-sensitivities calculated at 65 Pa CO_2. Thus, the more easily measured O_2-sensitivity confirmed trends seen in CO_2-sensitivity.

Table 2. Initial and total Rubisco activities, carbamylation level, and Rubisco protein levels in leaves of untransformed (UC82B), kanamycin-resistant (TCon-1), and SPS-transformed (SSU-9 and SSU-11) tomato lines grown at 35 or 65 Pa CO_2. Carbamylation level was calculated as: (initial/total) activities ×100. Tissue was sampled at 40 DAS in the middle of the light period; 10 leaves were sampled per plant. Values represent the mean±SE; n=3–4 plants per treatment

Grown at	Line	Rubisco activity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		Carbamylation	Protein
		Initial	Total	(%)	($\mu mol \cdot m^{-2}$)
35 Pa CO_2	UC82B	30±4	44±5	69±5	3.1±0.6
	Tcon-1	36±8	49±14	74±6	3.8±0.3
	SSU-9	40±3	56±5	72±2	2.5±0.2
	SSU-11	32±2	49±4	66±4	2.5±0.1
65 Pa CO_2	UC82B	44±6	62±6	70±4	3.4±0.1
	Tcon-1	25±1	35±3	71±4	3.3±0.4
	SSU-9	35±2	46±3	77±4	2.5±0.4
	SSU-11	40±7	53±5	74±7	3.1±0.2

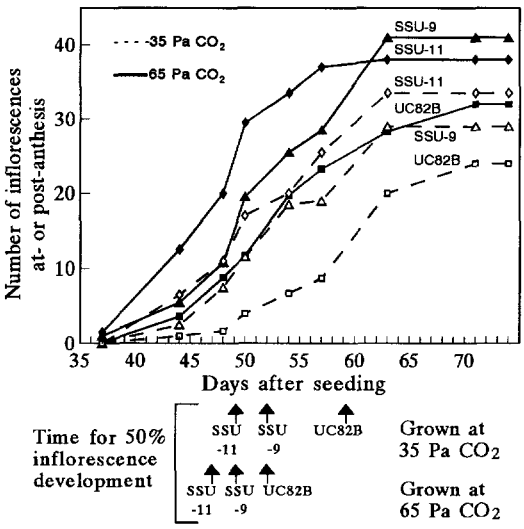

Fig. 4. Development of inflorescences over time for untransformed (*UC82B*) and SPS-transformed (*SSU-9* and *SSU-11*) tomato lines grown at 35 or 65 Pa CO_2 and 1000 µmol photons·m^{-2}·s^{-1}. The data represent inflorescence numbers per plant. *Arrows* at the bottom of the figure indicate the point at which 50% of the inflorescences had developed for each tomato line. Data are cumulative. Values represent the mean; n=3 plants per treatment. Standard errors are not shown for clarity

There were no differences in Rubisco parameters among any of the lines when grown at either 35 or 65 Pa CO_2 except for line Tcon-1, which had lower initial and total activities at 65 Pa CO_2 (Table 2).

Flowering and fruiting. The SPS transformants flowered earlier than line UC82B when grown at either 35 or 65 Pa CO_2 (Fig. 4). The effect was most pronounced for plants grown at 35 Pa CO_2, where the time to 50% blossoming was reduced by 7 (line SSU-9) and 10 (line SSU-11) d. High CO_2 also reduced the time to flowering for all lines, and the effect was greatest for the control line UC82B (7-d reduction for 50% inflorescence development). The final inflorescence number of the SPS

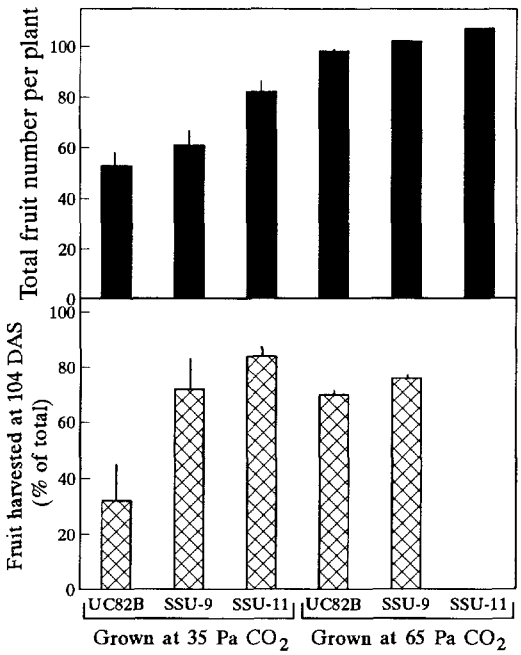

Fig. 5. The total final number of fruit harvested per plant and the proportion of final fruit number ready to harvest at 104 DAS (1st harvest) for untransformed (UC82B) and SPS-transformed (SSU-9 and SSU-11) tomato lines. Plants were grown at 35 or 65 Pa CO_2 and 1000 µmol photons·m^{-2}·s^{-1}. Values represent the mean±SE; n=3 plants per treatment

transformants was significantly greater than that of line UC82B when grown in either 35 (up to 1.4 times) or 65 (up to 1.3 times) Pa CO_2. In a separate experiment, the final number of inflorescences for the SPS transformants was greater than that of the control line TCon-1 when grown in either 35 (1.3 times) or 65 (1.5 times) Pa CO_2 (data not shown); the time of flowering was also reduced for the SPS-transformed lines compared to line TCon-1. The number of blossoms per inflorescence did not differ between lines (data not shown).

The total number of fruit produced was also significantly greater for the SPS transformants compared to line UC82B when grown at 35 Pa CO_2 (Fig. 5). The SPS-transformed lines produced up to 1.5 times the fruit of line UC82B (significant at the 5% level). The fruit number was not significantly different between lines when grown at 65 Pa CO_2, but growth at high CO_2 significantly increased fruit number for all lines. The percent of total fruit number ready to harvest at 104 DAS (first harvest) was also significantly greater for the SPS transformants compared to line UC82B when grown at 35 Pa CO_2 (Fig. 5). Interestingly, line SSU-11 grown at 65 Pa CO_2 had no mature fruit at 104 DAS (Fig. 5). In a separate experiment, the SPS-transformed lines produced 1.3 times more fruit (P<0.05) than line TCon-1 when grown at 35 Pa CO_2, and maturity was hastened for the SPS transformants (data not shown). The average individual fruit weights did not differ between the controls and SPS transformants (data not shown).

The SPS-transformed lines also had higher fresh and dry fruit weights compared to line UC82B when grown at 35 Pa CO_2 (Fig. 6). The increase for line SSU-11 was

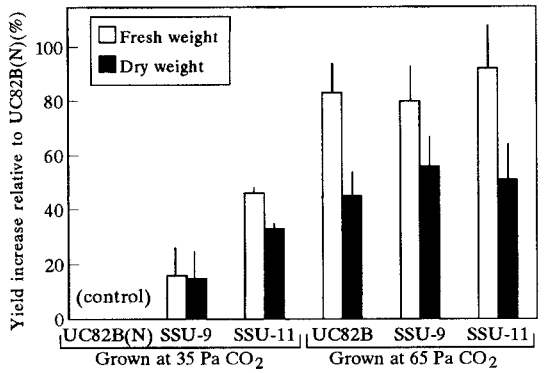

Fig. 6. The increase in the final fruit weight (fresh and dry) per plant for untransformed (UC82B) and SPS transformed tomatoes (SSU-9 and SSU-11) relative to UC82B(N) (grown at 35 Pa CO_2). Plants were grown at 35 or 65 Pa CO_2 and 1000 µmol photons·m^{-2}·s^{-1}. The final fruit weights per plant for UC82B(N) were 2394±128 g (fresh weight) and 143±7.7 g (dry weight). Values represent the mean±SE; n=3 plants per treatment

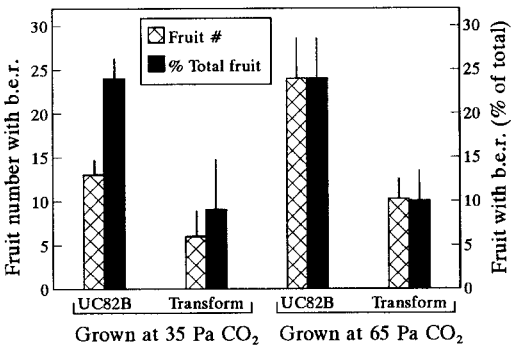

Fig. 8. The total fruit number per plant and proportion of total fruit with blossom-end rot (*b.e.r.*) for untransformed (UC82B) and SPS-transformed (SSU-9 and SSU-11) tomato lines. The data for lines SSU-9 and SSU-11 were combined since no significant differences were found between them. Plants were grown at 35 or 65 Pa CO_2 and 1000 µmol photons·m^{-2}·s^{-1}. Values represent the mean±SE; n=3 plants per treatment

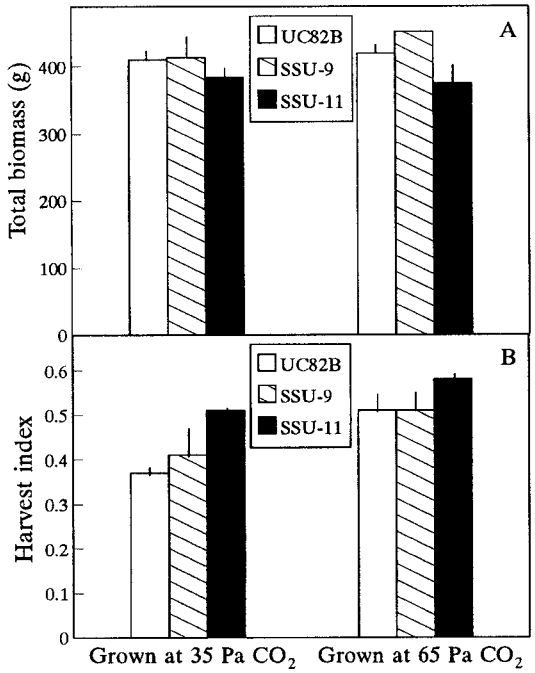

Fig. 7A, B. The total final dry weights (roots+shoots+fruit) per plant (**A**) and the final harvest index [(dry weight fruit)/(dry weight shoots+fruit)] (**B**) for untransformed (UC82B) and SPS-transformed (SSU-9 and SSU-11) tomato lines grown at 35 or 65 Pa CO_2. A light level of 1000 µmol photons·m^{-2}·s^{-1} was used during growth. Values represent the mean±SE; n=3 plants per treatment

statistically significant. No significant differences were found between lines when grown at 65 Pa CO_2. Growth at high CO_2 significantly increased fruit yield for all lines. The ratio of dry weight to fresh weight was less for fruit grown at 65 Pa CO_2. In a separate experiment, the SPS-transformed lines had a final fruit dry weight which was 1.7 times that of line TCon-1 when grown at 35 Pa CO_2 (data not shown).

The total final dry weights (roots+shoots+fruit) were not significantly different between treatments (Fig. 7A). The final harvest index for the SPS transformants was greater than line UC82B when plants were grown at 35 Pa CO_2 (Fig. 7B). The difference between lines SSU-11 and UC82B was statistically significant. The harvest index for line SSU-11 (grown at 35 Pa CO_2) was similar to the values for plants grown at 65 Pa CO_2. Line SSU-11 (grown at 65 Pa CO_2) had the highest harvest index. In a separate experiment, it was found that the SPS-transformed lines had a harvest index 1.5 times that of line TCon-1 when grown at 35 Pa CO_2 (data not shown).

The total fruit number and proportion of total fruit with blossom-end rot were significantly reduced (P<0.05) in the SPS-transformed lines when grown at either 35 or 65 Pa CO_2 (Fig. 8).

Discussion

Leaf SPS activities and assimilate partitioning. Leaf SPS activities of the SPS transformants were significantly greater than the controls for all treatments (Fig. 1). Activities were determined using both limiting and saturating concentrations of substrates; it is believed that the limiting assay is more representative of SPS activity in vivo (Huber and Huber 1992). In conjunction with the increased leaf SPS activities, there was a shift in carbon partitioning in favor of sucrose in both SPS-transformed lines (Table 1). Sonnewald et al. (1992) showed that tobacco and potato transformed with a spinach SPS clone contained two- to threefold more SPS protein but sucrose synthesis was not enhanced. By comparing the limiting and V_{max} assays, these workers showed that most of the excess SPS protein was deactivated in the tobacco and potato.

At 50 DAS, a decrease in leaf SPS activity was found for the SPS transformants grown in 65 Pa CO_2 relative to those grown at 35 Pa CO_2 (Fig. 1B). It was also found that Rubisco levels had dropped at 50 DAS for plants grown at high CO_2 (data not shown). Since the maize SPS is under the control of the SSU promoter, it is proposed that factors controlling Rubisco activity in tomato also affect expression of the maize SPS construct.

We found SPS activities in the transformed plants up to 3 times greater than found by Galtier et al. (1993). Our light levels (1000 μmol photons·m^{-2}·s^{-1}) were substantially higher than those of Galtier et al. (1993; 160 μmol photons·m^{-2}·s^{-1}). If expression of the SPS constructs was dependent on factors controlling Rubisco protein levels such as light, this could explain the reported differences in SPS.

Photosynthetic responses. An increased capacity for sucrose synthesis did not affect photosynthesis when plants were grown in 35 Pa CO_2 (Fig. 2A). This is similar to results obtained by Galtier et al. (1993). Control lines (UC82B and TCon-1) grown at 65 Pa CO_2 showed an acclimation to CO_2 such that the rates of photosynthesis measured at high CO_2 levels reached a plateau at a lower level than control plants grown at 35 Pa CO_2 (Fig. 2B). The acclimation response of the controls to high CO_2 resulted in photosynthetic rates which were lower than would be predicted from plants grown at 35 Pa CO_2 (Fig. 2A). Other workers have also shown that photosynthesis in tomato and other species acclimates to lower levels when grown at high CO_2 (Nilsen et al. 1983; Sage et al. 1989; Yelle et al. 1989; Besford et al. 1990). In contrast, SPS transformants grown at 65 Pa CO_2 had rates of photosynthesis significantly greater than the two control lines (Fig. 2B). The pattern of the curve for the SPS-transformed lines was similar to that for plants grown in 35 Pa CO_2 (compare Fig. 2A, B). These data indicate that an increased capacity for sucrose biosynthesis eliminated the normal acclimation response of tomato to high CO_2.

The SPS transformants grown at 65 Pa CO_2 also had a higher O_2-sensitivity of photosynthesis than line UC82B (Fig. 3), and they did not become CO_2-insensitive like the control lines (Fig. 2B). Insensitivity to O_2 and CO_2 is diagnostic of an end-product limitation on photosynthesis (Sharkey 1990). These data indicate that the SPS-transformed lines had a reduced limitation on photosynthesis imposed by end-product synthesis. Also, the leaf Rubisco levels of the untransformed control plants did not differ from the SPS transformants when grown at 65 Pa CO_2 (Table 2). Gas-exchange experiments have demonstrated that end-product synthesis can limit photosynthesis at high CO_2 levels, whereas Rubisco is not limiting for photosynthesis under these conditions (Sharkey 1990).

Relationships among photosynthesis, biomass accumulation, and yield. No differences in photosynthetic rates were found between lines when grown at 35 Pa CO_2. These data confirm the findings of Galtier et al. (1993) who found no differences in growth rate and photosynthesis of the SPS transformants prior to flowering in plants grown in 35 Pa CO_2. Differences in leaf photosynthesis were found in leaves grown and assayed at 65 Pa CO_2 (Fig. 2), but the total, final biomass was the same in all treatments (Fig. 7A). This could reflect the determinate nature of growth of line UC82B. It would be worthwhile to examine the effect of the SPS gene in indeterminate tomato cultivars. There was also no relationship between leaf photosynthesis and harvestable yield (Figs. 2, 6), which has been found in many studies (Nelson 1988).

Flowering and fruiting. Our results indicate that an increased capacity for sucrose synthesis may affect flowering in tomato. There was a reduction in the time to flowering and an increase in total flower production in the SPS-transformed lines (Fig. 4) when grown at either 35 or 65 Pa CO_2. Growth at high CO_2 also enhanced flowering for all lines (Fig. 4), which has been reported for tomato by other workers (Hickleton and Jolliffe 1978; Mortenson 1987; Yelle et al. 1990). The effect of the SPS gene on flowering was most pronounced at 35 Pa CO_2, even though rates of photosynthesis and growth were not enhanced in this condition. These data give evidence that flowering is not strictly coupled to the rate of photosynthesis and growth in tomato.

The enhanced flowering of the SPS-transformed lines did result in increased and earlier fruit production when plants were grown at 35 Pa CO_2 (Figs. 5, 6). There was also a significant increase in flowering for the SPS transformants grown at 65 Pa CO_2 relative to the control line (Fig. 4) but yield was not significantly increased (Fig. 6). Line SSU-11 grown at 65 Pa CO_2 was the most advanced early in development (Fig. 4), but at the time of fruit harvest it was the least advanced (Fig. 5). It is possible that an imbalance occurred between the amounts of foliage and fruit, reducing the overall potential for whole-plant photosynthesis. Indeed, this treatment had the highest harvest index (Fig. 7B).

The flowering and fruiting of the SPS-transformed lines grown at 35 Pa CO_2 showed some similarities to line UC82B grown at high CO_2 (Figs. 4–6). In both cases the tomato plant may be experiencing an increased sucrose supply during the day, either by a shift in partitioning in favor of sucrose in the SPS transformants, or by an increase in photosynthetic rate when grown at 65 Pa CO_2. Ho (1977) has shown that carbon translocation increases when tomato plants are grown in high CO_2. Although the SPS transformants may transport more carbon during the day, total sucrose production per unit of leaf area for plants grown at 35 Pa CO_2 should not differ since photosynthetic rates were the same (Fig. 2) and carbon will be transported to sink tissue as sucrose, regardless of whether it was originally incorporated into sucrose or starch. Therefore, some diurnal component may be involved in the flowering and fruiting responses found in this study. Differences in growth conditions during the light and dark periods could be involved in these interactions.

The incidence of blossom-end rot was reduced in the SPS-transformed lines. Ho et al. (1993) indicated that competition between the shoot and fruit for available Ca^{2+} can affect blossom-end rot. However, the reduced blossom-end rot in the SPS-transformed tomatoes is unlikely a direct result of the altered whole plant carbon partitioning, since no strict correlations were found between harvest index and the incidence of blossom-end rot (Figs. 7B, 8). The reason for the reduced blossom-end rot is unclear.

Summary. The SPS-transformed plants had a greater capacity for sucrose synthesis in their leaves, and a reduced end-product limitation on photosynthesis. Photosynthesis

of the SPS transformants did not show the normal acclimation response which occurs when tomato is grown in high CO_2, resulting in a higher rate of photosynthesis for the SPS-transformed lines. Flowering was enhanced in the SPS-transformed lines, providing direct evidence that altering sucrose synthesis can affect flowering in tomato. Enhanced flowering resulted in increased and earlier fruit yields when plants were grown at 35 Pa CO_2 . The incidence of blossom end rot was also reduced in the transformants. However, since carbon partitioning is influenced greatly by the environment, it is difficult to predict whether these effects on flowering and fruiting will be found under all growth conditions.

This research was supported by U.S. Department of Energy grant FG02-87ER13785. B.J.M. thanks the Natural Sciences and Engineering Research Council of Canada for financial support. We are grateful to Toni A. Voelker (Calgene Inc.) for supplying tomato seeds and valuable advice.

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