Elevated sucrose-phosphate synthase activity in transgenic tobacco sustains photosynthesis in older leaves and alters development

Charles J. Baxter1*, Christine H. Foyer2, Janice Turner2, Stephen A. Rolfe1 and W. Paul Quick1

1 Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK
2 Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

Received 17 December 2002; Accepted 16 April 2003

Abstract

Constitutive over-expression of a maize sucrose-phosphate synthase (SPS) gene in tobacco (Nicotiana tabacum) had major effects on leaf carbohydrate budgets with consequences for whole plant development. Transgenic tobacco plants flowered earlier and had greater flower numbers than wild-type plants. These changes were not linked to modified source leaf carbon assimilation or carbon export, although sucrose to starch ratios were significantly higher in leaves expressing the transgene. The youngest and oldest leaves of plants over-expressing SPS had up to 10-fold wild-type maximal extractable SPS activity, but source leaf SPS activities were only 2–3 times greater in these lines than in the wild type. In the oldest leaves, where the expression of the transgene led to the most marked enhancement in SPS activity, photosynthesis was also increased. It was concluded that these increases in the capacity for sucrose synthesis and carbon assimilation, particularly in older leaves, accelerate the whole plant development and increase the abundance of flowers without substantial changes in the overall shoot biomass.

Key words: Carbon partitioning, flowering, photosynthesis, plant development, sucrose-phosphate synthase.

Introduction

Sucrose is the major transport carbohydrate in a range of plant species (Farrar, 1996) and, as such, forms the interface between carbon assimilation in source tissue and carbon utilization in sink tissue. The abundance of sucrose in the source leaf is a determinant of the rate of sucrose export from the leaf (Ho and Thornley, 1978). Sucrose and its metabolites are also effectors of gene expression; hence the concentration of sucrose may play a part in determining the expression of genes associated with a number of metabolic pathways (Sheen, 1990; Krapp and Stitt, 1995; Koch, 1996). In addition, as the major sink for triose phosphate exported from the chloroplast, sucrose synthesis may directly influence the rate of photosynthesis (Stitt, 1986). The rate of sucrose synthesis is correlated with the rate of photosynthesis (Battistelli et al., 1991) and also with the rate of export from leaves (Rocher et al., 1989).

Sucrose-phosphate synthase catalyses a reversible reaction in the pathway of sucrose synthesis, the formation of sucrose-6-phosphate from fructose-6-phosphate (Fru6P) and UDPGlucose (UDPGlc) (Leloir and Cardini, 1955). It catalyses an essentially irreversible reaction in vivo due to its close association with the enzyme sucrose phosphate phosphatase (Echeverria et al., 1997). SPS is subject to a complex system of regulation involving post-translational modulation of activity via protein phosphorylation (Walker and Huber, 1989; Huber and Huber, 1996) and direct control via metabolic effectors (Siegl and Stitt, 1990). The SPS reaction has been identified as a key step in the control of sucrose synthesis (Stitt and Quick, 1989).

The effects of SPS expression on photosynthesis and leaf carbohydrate metabolism have been studied in several species (Worrell et al., 1991; Galtier et al., 1993, 1995; Signora et al., 1998). This has led to a greater understanding of the role of SPS in the regulation of these processes. For example, the source leaves of plants over-expressing...
SPS have increased leaf sucrose-to-starch ratios and, in some circumstances, exhibited higher maximal photosynthetic rates (Galtier et al., 1993, 1995; Micallef et al., 1995; Signora et al., 1998; Ono et al., 1999). In addition, there is evidence that tomato plants expressing an SPS gene under the control of the CaMV35S promoter had increased biomass compared with controls (Foyer and Ferrario, 1994; Laporte et al., 1997).

To date, much of the work carried out on SPS over-expression has concentrated on effects on source leaf metabolism. However, SPS over-expression has the potential to cause profound effects on developmental processes such as flowering (Micallef et al., 1995), fruiting (Nguyen-Quoc et al., 1999) and the sink-to-source transition (Cheng et al., 1996). In addition, source capacity has been shown to be an important determinant of the phase of tobacco development (Tsai et al., 1997). In the present study transgenic tobacco plants over-expressing a maize SPS cDNA were used to examine the relationship between elevated SPS activity and carbon assimilation and metabolism in all leaves (young, mature and senescent) in relation to flower production and shoot biomass. It is shown here that the transgenic plants exhibiting increased rates of SPS activity (Baxter et al., 2001) have accelerated flower development and increased flower numbers. These important traits may be related to modified leaf carbohydrate metabolism in the youngest and oldest leaves rather than the source leaves.

Materials and methods

Plant growth conditions

Transgenic tobacco plants, in which increased SPS activity was previously described (Baxter et al., 2001), were grown in growth cabinets with artificial lighting providing growth irradiances of 800 μmol m⁻² s⁻¹ in a 16/8 h light/dark regime. In addition, where larger numbers of plants were required for analysis, plants were grown in greenhouses with supplementary lighting providing a growth irradiance of 250–400 μmol m⁻² s⁻¹ in a 16/8 h light/dark regime. In addition, where larger numbers of plants were required for analysis, plants were grown in greenhouses with supplementary lighting providing a growth irradiance of 250–400 μmol m⁻² s⁻¹. The data described in Tables 1 and 2 were obtained from a T1 population of greenhouse-grown plants. All other experiments were carried out on two homozygous lines selected for elevated SPS activity (T2 generation). Individual tobacco plants were grown in large round pots (20 cm diameter) containing compost (Levingtons M3; 6 kg pot⁻¹) supplemented with Osmocote slow release fertilizer (30 g pot⁻¹). In experiments where measurements were made on individual leaves 6-week-old plants were used. These plants possessed up to 14 leaves below a rosette of 3–5 very small leaves at the top of the plant.

Carbon partitioning in leaf discs

Leaf discs (1.1 cm²) were cut from each of the first three fully expanded source leaves and placed in an oxygen electrode leaf chamber on a wire gauze. Leaf discs were placed above moistened matting containing 250 μl of a labelled solution of 14CO2 (2 M KHCO3, 2 M K2CO3 containing 1.25 MBq 14CO2 ml⁻¹) and illuminated for 20 min at an irradiance of 500 μmol m⁻² s⁻¹ at 25 °C. Following incubation, leaf discs were heated in 80% (v/v) ethanol at 70 °C for 1 h to kill the leaf tissue and extract soluble carbohydrate. The ethanol-soluble fraction was dried in a vacuum oven, resuspended in 1 ml of distilled water and subjected to ion exchange chromatography (Quick et al., 1989). Acidic, neutral and basic fractions were then counted on a liquid scintillation counter. Radioactivity in the insoluble fraction was determined by homogenizing the ethanol-insoluble fraction and counting aliquots on a liquid scintillation counter.

Protein extraction for SPS assay

Frozen leaf samples were ground in liquid nitrogen with 1 mg of insoluble PVPP (polyvinyl polypyrrolidone) and 4 vols (w/v) of extraction buffer (50 mM HEPES-KOH pH 7.5, 10 mM MgCl2, 1 mM EDTA, 2 mM DTT (dithiothreitol), 1 mM PMSF (phenylmethylsulphonyl fluoride), 1 mM benzamidine, 5 mM 3-aminoo-n-caproic acid, 0.1% (v/v) Triton X-100, and 10% (v/v) glycerol). Samples were allowed to thaw, transferred to chilled Eppendorf tubes and centrifuged at 4 °C, 12,000 g for 2 min. An aliquot of the resulting supernatant was then desalted on a NAP 5 column (Pharmacia biotech, Sephadex G25 medium), pre-equilibrated with ice-cold extraction buffer minus Triton X-100 and PVPP. Extracts were collected in pre-chilled Eppendorf tubes and stored in liquid nitrogen prior to analysis.

Sucrose-phosphate synthase assay

SPS activity was assayed by quantifying the fructosyl moiety of sucrose using the anthrone test (Lunn and Furbank, 1997). Samples were incubated for 20 min at 25 °C in 200 μl of buffer (50 mM HEPES-KOH pH 7.5, 20 mM KCl, and 4 mM MgCl2) containing (a) Vmax assay; 12 mM UDPGlc and 10 mM Fru6P (in a 1:4 ratio with glucose-6-phosphate (Glc6P)) and (b) Vlimiting assay; 4 mM UDPGlc and 2 mM Fru6P (in a 1:4 ratio with Glc6P) and 5 mM KH2PO4. The reaction was stopped by incubation at 95 °C for 5 min and samples were centrifuged at 4 °C, 12,000 g for 5 min. 100 μl of the resulting supernatant was added to 100 μl of 5 M KOH and incubated for a further 10 min at 95 °C to destroy any unreacted hexose phosphates. After adding 4 vols of 0.14% (w/v) anthrone reagent (14.6 M H2SO4) absorbance was measured at 620 nm. A standard curve with 0–200 nmol sucrose was used to calculate absolute amounts of sucrose-6-P made during incubations. Reactions containing boiled protein extract or with hexose phosphates omitted were included to provide blank values.

Carbohydrate assays

Carbohydrates were assayed using a method modified from Caporn et al. (1999). Leaf discs were cut from each leaf, placed immediately in a glass test tube containing 80% ethanol (v/v) and incubated at 70 °C for 2 h in a heating block (Technic Instruments) to extract soluble carbohydrates. Leaf discs in ethanol were then stored at –80 °C in stoppered test tubes prior to carbohydrate analysis.

Starch: Extracted leaf discs were ground in a glass tissue homogenizer in 1 ml of distilled water and then autoclaved for 30 min at 121 °C, 0.2 MPa. A 50 μl aliquot of this solution was added to 150 μl of MES (2-morpholinoethanesulphonic acid) buffer (500 mM MES, pH 4.5), 50 μl of amyloglucosidase (0.28 units μl⁻¹) and 50 μl of α-amylase (0.4 units μl⁻¹) and incubated at 37 °C for 5 h. Following incubation, the samples were centrifuged at 23,000 g for 2 min and a 50 μl aliquot of the supernatant was taken for assay of the total leaf starch (see glucose assay below).

Soluble carbohydrate: The fraction containing soluble carbohydrate (80%, v/v, ethanol solution) was dried overnight in a vacuum oven at 40 °C and the residue redissolved in 1 ml of distilled water. Samples were stored at –20 °C prior to enzymatic assay.
Enzymatic assay of carbohydrates

Assay of carbohydrates was carried out on a plate reader (A-5022 Anthos HtIII, Salzburg). Plates were loaded with 50 μl of extract (soluble fraction or starch), 160 μl of HEPES buffer (100 mM HEPES pH 7.5, 5 mM MgCl2), 10 μl NAD (40 mM), 10 μl ATP (100 mM), and 0.5 units of Glc6P dehydrogenase. The enzymes hexokinase (glucose assay, 0.5 units), phosphoglucomutase, phosphoglucomutase isomerase (fructose assay, 0.6 units), and invertase (sucrose assay, 8 units) were then sequentially added and the change in absorbance at 340 nm related to measured standards.

Measurement of gas exchange and rates of photosynthesis

Gas exchange measurements were carried out using a portable CO2 and H2O infrared gas analyser (IRGA-LCA3) (ADC, Hertfordshire, UK) and a PLC-4 Parkinson-type leaf chamber (ADC, Hertfordshire, UK). Measurements were carried out under conditions of ambient CO2, supplied via an airline and adjusted to approximately 350 ppm within the IRGA. A Schott lamp was used to supply a constant irradiance of 800 μmol m\(^{-2}\) s\(^{-1}\) and a CO2 concentration of 350 ppm in the gas phase. A 10 min chase using 2 ml unlabelled NaH14CO3 (0.74 MBq) (Amersham) was added at a rate of 0.2 ml min\(^{-1}\). The main air supply was then sequentially added and the resulting CO2-free air bubbled in a glass vial containing 5 ml 100% lactic acid. Over a period of 5 min, 1 ml 31.5 mM NaHCO3 combined with 10 μl NaH14CO3 (0.74 MBq) (Amersham) was added at a rate of 0.2 ml min\(^{-1}\) (Pharmacia LKB PumpII) into the vial containing lactic acid using a peristaltic pump, liberating 14CO2 at a final concentration of 350 ppm in the gas phase. A 10 min chase using 2 ml unlabelled 31.5 mM NaHCO3 followed. The main air supply was then reconnected directly to the chamber and efflux of 14C from the leaf was monitored over a period of 5 h using the Geiger–Muller tube connected to a Geiger counter (Mini Instruments, London, UK) and a chart recorder (Rikadenki Mitsui Electronics Ltd., UK). The radioactivity associated with tobacco leaves was expressed as the percentage of initial 14C fixed remaining in the leaf. The size of the labile sucrose pool and the rate constant for movement from this pool were subsequently calculated according to the model of Rocher and Prioul (1987) using the kinetics of the radioactivity monitored during the chase period, and data relating to photosynthetic rate and the partitioning of photosynthate to sucrose obtained from the experiments described.

Statistical analysis

Statistical analyses were carried out using t-tests and ANOVAs (Minitab v11) and regression analysis (Sigma plot v4.0). A 5% probability was considered significant.

Results

Experimental design

The data presented in this paper were obtained from experiments carried out on two populations of transgenic tobacco plants over-expressing a maize SPS cDNA. Large-scale growth and physiological studies were conducted in the greenhouse on plants grown to maturity. More detailed characterizations of individual leaf enzyme activities and photosynthetic parameters were undertaken on plants from growth cabinets grown at an irradiance of 800 μmol m\(^{-2}\) s\(^{-1}\). All comparisons of individual leaves were made on 6-week-old wild-type and transgenic plants, just prior to flowering in these growth conditions. At this stage the height of the transgenic and wild-type plants was similar and all plants exhibited the same number of leaves. Measurements of CO2 assimilation rates were made on individual leaves illuminated at the growth irradiance. Following photosynthetic measurements, the leaf tissue was harvested for protein extractions and the measurement of carbohydrate.

Plant growth

Tobacco plants expressing the SPS transgene had a significantly greater number of flowers than wild-type plants (Fig. 1A). These plants also flowered significantly earlier than the wild type (Fig. 1B). The total plant dry weights measured following seed set tended to be greater in transgenic lines 12-2 and 11-3 than wild-type plants (Fig. 1C). However, these increases in biomass were not statistically significant (P >0.05 ANOVA).

Carbon partitioning in source leaves

Rates of CO2 fixation in fully expanded source leaves determined by the incorporation of 14CO2 did not correlate with SPS activity (Table 1). However, increasing SPS activity altered the abundance of 14C present in the neutral and insoluble fractions such that there was an increase in partitioning to sucrose (Table 1). This was achieved at the expense of partitioning to pathways of starch synthesis and not as a result of increased rates of carbon assimilation (Table 1).

Export of sucrose from source leaves

Comparable patterns of export were observed in SPS over-expressors and wild-type plants, moreover, the increased rates of SPS activity did not significantly influence the rate of sucrose export from transgenic leaves (Table 2). However, these data show that the size of the pool for sucrose export was greater in transgenic plants expressing the highest activities of SPS (Table 2).

SPS activity and leaf ontogeny

In wild-type plants SPS activity (V\(_{\text{max}}\)) was greatest in the fully expanded source leaves (leaves 6–8) when compared
with either young, developing leaves or the oldest leaves (Fig. 2). This age-dependent pattern of SPS activity was much less apparent in transgenic plants. SPS activity was always significantly greater in the leaves of transgenic plants compared with the equivalent leaves of the wild-type plant. This overall increase in SPS activity and reduction in age-dependent expression led to the fold-increase in SPS activity being greatest in the youngest and, particularly, oldest, leaves of transgenic plants with values being approximately 5× that of the wild type.

Increases in maximal extractable SPS activity were mirrored by changes in rates of SPS activity measured in a limiting assay (Fig. 3A), however, changes in \( V_{\text{limiting}} \) activity were even more pronounced with leaf age. The percentage activation of SPS (\( V_{\text{limiting}}/V_{\text{max}} \) activity) fluctuated with leaf age in all lines (Fig. 3B), but was greater in the SPS over-expressors than in the wild-type plants. This suggests that the product of the maize SPS transgene was subject to less post-translational regulation of activity in transformed plants. This agrees with data on the kinetic properties of the enzyme described previously (Baxter et al., 2001).

**Leaf carbohydrate contents**

Sucrose:starch ratios varied with leaf age in the wild type, being greater in younger expanding and older leaves than in source leaves (Fig. 4A, closed symbols). The sucrose:starch ratio in transgenic leaves also fluctuated with leaf age, however, in this case changes in the abundance of these carbohydrates were greater (Fig. 4A). In the source leaves of these plants sucrose:starch ratios were up to twice those of the wild type (Fig. 4A, leaves 6–8). In the younger expanding leaves of the transgenic plants the carbohydrate content was not significantly greater than the wild type, although there was a trend to increasing sucrose in these tissues (Fig. 4A, leaves 9–13). In the older transgenic leaves sucrose:starch ratios were increased to a greater degree than those observed in the wild type (Fig. 4A, leaves 1–5).

**Table 1. The rate of \(^{14}\)CO\(_2\) fixation and the proportion of leaf \(^{14}\)C in starch (insoluble fraction) or sucrose (neutral fraction)**

Measurements of CO\(_2\) fixation and carbon partitioning were calculated from \(^{14}\)CO\(_2\) feeding experiments carried out on leaf tissue harvested from plants during the first 4 h of the photoperiod. Values represent the mean ± SE; \(n=3\)–5 plants per group.

<table>
<thead>
<tr>
<th></th>
<th>SPS activity ((\mu)mol m(^{-2}) s(^{-1}))</th>
<th>Rate of CO(_2) fixation ((\mu)mol m(^{-2}) s(^{-1}))</th>
<th>% (^{14})C neutral fraction</th>
<th>% (^{14})C insoluble fraction</th>
<th>Ratio of neutral:insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>2.11 (0.28)</td>
<td>15.99 (1.18)</td>
<td>58.66 (1.15)</td>
<td>29.63 (3.1)</td>
<td>0.56 (0.04)</td>
</tr>
<tr>
<td>Medium expressor</td>
<td>4.65 (0.52)</td>
<td>16.16 (1.48)</td>
<td>52.56 (1.81)</td>
<td>30.83 (3.39)</td>
<td>0.60 (0.08)</td>
</tr>
<tr>
<td>High expressor</td>
<td>8.14 (0.41)</td>
<td>15.46 (2.42)</td>
<td>47.21 (0.72)</td>
<td>32.68 (3.49)</td>
<td>0.70 (0.08)</td>
</tr>
</tbody>
</table>

**Table 2. The export of \(^{14}\)C from leaves of wild-type and transgenic tobacco plants**

The rate of \(^{14}\)C export was measured from the leaves of wild-type plants and transformed lines. Leaves were fed with a pulse of \(^{14}\)CO\(_2\) for 5 min and the export of radioactivity from the leaf was monitored for the following 5 h of the photoperiod. Using the kinetics of the export data, photosynthetic rate and partitioning of photosynthate to sucrose, the size of the transport pool of carbon and the actual rate of carbon export from the leaf were calculated according to the model of Rocher and Prioul (1997).

<table>
<thead>
<tr>
<th></th>
<th>SPS activity (% wild type)</th>
<th>Transport pool size (g C m(^{-2}))</th>
<th>Export rate (g C h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>100</td>
<td>0.30</td>
<td>0.36</td>
</tr>
<tr>
<td>Medium expressor</td>
<td>140</td>
<td>0.29</td>
<td>0.25</td>
</tr>
<tr>
<td>High expressor</td>
<td>400</td>
<td>0.62</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Fig. 1. The total number of flowers per plant (A), the time to flowering (B) and the total shoot dry weight (C) of wild type (closed bars) and transgenic tobacco plants (open bars). Bars marked with asterisks denote significant differences from wild type plants (ANOVA, \(p<0.01\)).
To study the impact of SPS over-expression on the development of photosynthetic capacity, rates of photosynthesis were measured in leaves of different ages from wild-type and transgenic tobacco plants at 42 d after planting. The rate of photosynthetic carbon assimilation was relatively constant in younger leaves and in fully expanded leaves from wild-type plants (Fig. 2B, closed symbols). A similar relationship was found for the leaves of SPS over-expressors (Fig. 2B). Photosynthetic capacity decreased with increasing leaf age in both transgenic and wild type plants. However, the decline was greater in the leaves of wild-type plants, suggesting that an increased capacity for sucrose synthesis maintained the rate of photosynthesis in older leaves.

**Discussion**

Increases in SPS activity are associated with the transition from source to sink during leaf ontogeny (Walker and Huber, 1989). Moreover, increased SPS activity has been...
shown to modify sink capacity. For example, SPS over-expression led to an elevated rate of sucrose unloading into tomato fruit (Nguyen-Quoc et al., 1999). Constitutive enhancement of leaf SPS activity, therefore, has important implications for source:sink relationships as well as carbon metabolism. The significant increases in total SPS activity and in the activation-state of the enzyme achieved in the present study by constitutive expression of a maize cDNA in tobacco might therefore be predicted to influence source:sink relationships, as was observed. Since the enhancement of SPS activity was greatest in the youngest and oldest leaves, it is also possible to predict that any effect of SPS expression may be greatest in these leaves. Hence, enhanced SPS activity would have a positive effect on the overall capacity to assimilate and partition carbon. Evidence in support of this view is as follows.

SPS over-expressors had significantly more flowers and a decrease in time to flowering than the controls. However, tobacco plants have few clearly defined sinks (Hajirezaei et al., 1994) and hence a change in sink capacity is hard to demonstrate. A similar reduction in flowering time was observed in tomato fruit over-expressing SPS under the control of the rbcS promoter (Micallef et al., 1995). Plant growth and development respond to changes in the concentration of phloem sucrose (Corbesier et al., 1998). A trend towards increased shoot biomass in the SPS over-expressors was indicated, however, this relationship was not statistically significant in greenhouse experiments in which 10 plants were grown per line. Subtle increases in biomass may only be statistically apparent in much larger scale growth experiments. A correlation between leaf SPS activity and shoot biomass has previously been reported in transgenic tomato plants (Foyer and Ferrario, 1994; Laporte et al., 1997). The observed effects of SPS over-expression on the development of transgenic tobacco plants reported here must result from (1) increases in the capacity to assimilate carbon, (2) from changes in the partitioning of carbon or (3) from perturbations in both of these processes.

Increased carbon partitioning to sucrose, together with a decrease in partitioning to starch, accompanied higher SPS activities in fully expanded source leaves. These effects were independent of the rate of CO₂ fixation. The shift in source leaf carbon partitioning towards increased sucrose: starch ratios agrees with previous observations in tomato (Galtier et al., 1995; Micallef et al., 1995). The sucrose concentration of source leaves also influences the rate and direction of sucrose transport (Swanson and Christy, 1976; Ho and Thornley, 1978). Compartamental analysis of carbon efflux from the source leaves (6–8) of different tobacco lines suggested that there is an increase in the size of the transport carbohydrate pool in leaves expressing the transgene (Table 2). However, measured rates of sucrose export from the fully expanded source leaves were similar in transgenic plants and the wild type (Table 2). Similarly, no evidence for a role of sucrose concentration in controlling export rate was obtained in a starchless tobacco mutant (Huber and Hanson, 1992). This may be because the rate of phloem loading in tobacco is also determined by sucrose transporter activity (Burkle et al., 1998).

There is no evidence to suggest that the rate of carbon assimilation was increased as a result of high SPS activity in source leaves. This, together with the absence of SPS-dependent marked changes in carbon export from source leaves, would lead to the conclusion that the observed enhanced flowering in transgenic tobacco plants was not caused by the effects of high SPS in source leaves. The same cannot be said for their younger expanding counterparts or for the older leaves, since these organs exhibit far greater increases in SPS activity than the fully expanded source leaves of transgenic plants. Therefore, it was concluded that changes in the metabolism of these leaves have a significant effect on the capacity of the whole plant to assimilate carbon. The most striking evidence in support of this conclusion comes from measurements of photosynthetic rate. In younger and older leaves, where SPS activity was up to 10-fold greater than the wild type, rates of photosynthesis were much greater than their respective controls. The mechanism whereby high SPS activity stimulates photosynthesis is uncertain, but it probably occurs by preventing starch accumulation and associated repression of gene expression by high leaf carbohydrate contents. However, a comparable stimulation of photosynthesis was observed in tobacco plants supplied with sucrose (Furbank et al., 1997). Indeed, they also explained this effect in terms of modified gene expression. Alternatively, enhanced SPS activity may simply alleviate the back pressure on photosynthesis by removing triose-phosphate in conditions of high light and CO₂ availability (Stitt, 1986).

In addition to an enhanced rate of photosynthesis, significant increases in SPS activity in the youngest and oldest leaves were accompanied by higher leaf sucrose:starch ratios. While leaf sucrose:starch ratios are globally increased as a result of SPS over-expression, the proportionally greater change in these leaves suggests a major shift in their carbohydrate metabolism. In the older leaves source capacity appears to be maintained as a result of SPS over-expression, implying that senescence, as measured by physiological activity is delayed. The evidence presented here, suggesting a role for SPS in the source-sink relationships of younger and older tobacco leaves, is supported by similar observations by other authors on transgenic tobacco plants where the abundance of photosynthetic enzymes was decreased (Tsai et al., 1997; Olcer et al., 2001). Decreases in leaf ribulose-1,5-bisphosphate carboxylase/oxygenase or sedoheptulose-1,7-bisphosphate resulted in marked changes in plant development (Tsai et al., 1997; Olcer et al., 2001). Taken together, these observations and the results obtained with SPS trans-
formants reported here, confirm that source strength, as determined here by the capacity of enzymes such as SPS, is of major importance in governing plant development.

In conclusion, these results demonstrate the impact of increased SPS activity on plant development. Early flowering is an important trait and is frequently a criterion for selection in plant breeding programmes. Since no changes in either the photosynthetic CO₂ assimilation and export rates of source leaves could be detected in plants with constitutive high SPS expression, it was concluded that the proportionally greater increase in SPS activity in younger and older leaves influences these traits. Moreover, because the effect of high SPS activity was most marked on the oldest leaves it was concluded that sustained photosynthesis over a longer period in these leaves has a crucial impact on the capacity of the tobacco plant to generate new sinks.

Acknowledgements

This work was supported by the BBSRC (grant number PG50/586). CJ Baxter is grateful to the BBSRC and IGER, Aberystwyth for a BBSRC-CASE studentship.

References


Quick WP, Siegl G, Neuhaus HE, Feil R, Stitt M. 1989. Short-
term water stress leads to a stimulation of sucrose synthesis by activating SPS. *Planta* **177**, 536–546.


