

Regulation of Key Enzymes of Sucrose Biosynthesis in Soybean Leaves¹

Effect of Dark and Light Conditions and Role of Gibberellins and Abscisic Acid

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ABSTRACT

An important part in the understanding of the regulation of carbon partitioning within the leaf is to investigate the endogenous variations of parameters related to carbon metabolism. This study of diurnal changes in the activities of sucrose-synthesizing enzymes and levels of nonstructural carbohydrates in intact leaves of field-grown soybean plants (*Glycine max* [L.]) showed pronounced diurnal fluctuations in sucrose phosphate synthase (SPS) activity. However, there was no distinct diurnal change in the activity of fructose-1,6-bisphosphatase (F1,6BPase). SPS activity in leaves from plants grown in controlled environments presented two peaks during the light period. In contrast to field-grown plants, F1,6BPase activity in leaves from growth chamber-grown plants manifested one peak during the first half of the light period. In plants grown under both conditions, sucrose and starch accumulation rates were highest during early hours of the light period. By the end of the dark period, most of the starch was depleted. A pattern of diurnal fluctuations of abscisic acid (ABA) levels in leaves was also observed under all growing conditions. Either imposition of water stress or exogenous applications of ABA inhibited F1,6BPase activity. However, SPS-extractable activity increased following water deficit but did not change in response to ABA treatment. Gibberellin application to intact soybean leaves increased levels of both starch and sucrose. Both gibberellic acid (10^{-6} M) and gibberellins 4 and 7 (10^{-5} M) increased the activity of SPS but had an inconsistent effect on F1,6BPase. Correlation studies between the activities of SPS and F1,6BPase suggest that these two enzymes are coordinated in their function, but the factors that regulate them may be distinct because they respond differently to certain environmental and physiological changes.

One of the first branch points for the use of newly fixed carbon in soybean leaves is between starch and sucrose. The pathways of sucrose and starch formation are interdependent as they compete for the same pool of triose-P, generated by

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the Calvin cycle in the chloroplast. As a result of this complex interdependency, it appears that sucrose formation in the cytoplasm and starch synthesis in the chloroplast are reciprocally related (11). Thus, it seems that factors that regulate sucrose formation can also influence starch synthesis. Additionally, because sucrose is the dominant form of carbon transported to developing organs and is an important storage compound in higher plants, it is important to conduct further research on the biochemical regulation of sucrose biosynthesis. The mechanism of regulation of sucrose biosynthesis is still poorly understood. However, the activities of F1,6BPase³ (EC 3.1.3.11) and SPS (EC 2.4.1.14) are presently recognized as rate limiting and important in the regulation of the photosynthetic sucrose production pathway (27, 28).

The environmental and developmental signals that may regulate leaf carbohydrate synthesis and partitioning are yet to be fully understood. Diurnal fluctuations in SPS activity have been reported in soybean plants grown in a greenhouse environment (21). Diurnal changes of SPS activity in leaves of other species, such as maize, cotton, barley, spinach, and tobacco, have also been investigated (27).

In the present paper, we report results from investigations of diurnal fluctuations of SPS and F1,6BPase activities and carbohydrate levels (starch, sucrose, hexoses) in leaves of field- and growth chamber-grown plants. Furthermore, we examine the relationships among SPS, F1,6BPase, and leaf carbohydrate content under the above conditions. This could help reveal whether an apparent coordinate regulation of cytosolic F1,6BPase and SPS exists and, if so, permit investigation of the possible parameters that modulate this coordinate regulation of the two key enzymes of sucrose biosynthesis.

The movement of photoassimilate from source to sink can be regulated at numerous levels. A close examination of the various components involved in the overall process of partitioning indicates that plant hormones may serve as modulators of many of the rate-limiting components (2). Sink removal in grape plants resulted in a decrease in leaf levels of

³ Abbreviations: F1,6BPase, fructose-1,6-bisphosphatase; GA, gibberellin; SPS, sucrose phosphate synthase; UDP-G, UDP-glucose; GA_{4/7}, gibberellins 4 and 7; F2,6BP, fructose-2,6-bisphosphate; F-6-P, fructose-6-phosphate.

GA-like substances; this could lead us to suggest that sinks could export GAs to source leaves (9). Moreover, it has been documented that developing sinks may control the levels of ABA in source leaves of soybean plants (24). There have also been reports of a rapid change in SPS activity that occurs in response to source-sink manipulation (13). In girdled leaves, SPS activity decreased by 50 and 75% after the phloem was blocked for 3 and 24 h, respectively (13).

This evidence provides the basis for the hypothesis that sink-produced or sink-exported plant hormones could play an essential role in the rate of carbohydrate synthesis and partitioning in source leaves and could act as regulators of the activities (or synthesis) of key enzymes in sucrose biosynthesis (SPS and F1,6BPase). GAs and ABA have been reported to influence carbohydrate transport out of leaves of tomato and bean plants (1, 5). There have also been indications that, in excised leaf systems of beans and sugar beet, ABA and GA₃ modified the activity of SPS (7). To date, however, it is still unclear how these two plant hormones affect the whole process of carbohydrate partitioning in soybean leaves, and, specifically, their role in the sucrose biosynthetic pathway is still subject to further investigations. Objectives of the present study were to determine the effect of exogenous applications of GAs and the increase in endogenous leaf levels of ABA on carbon partitioning between starch and sucrose within the leaf and to investigate the role of these growth substances in the regulation of activities of key sucrose-metabolizing enzymes.

MATERIALS AND METHODS

Plant Material and Chemicals

Soybean plants (*Glycine max* [L.] Merr. cv Evans) were grown from seeds, planted in a 1:1:2 mixture (v/v) of sand:soil:peat in 10-cm plastic pots. Unless specified otherwise, growth chambers were maintained at 26/19°C (day/night) temperature, and a 14-h photoperiod, with a PPFD at the top of the canopy of 400 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by a combination of cool-white fluorescent lamps (F48T12/CW/WHO Philips bulbs) and incandescent lamps (60-W Philips bulbs) or 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in a growth chamber containing a mixture of metal halide and sodium vapor lamps. Although not controlled, the CO₂ level was 350 $\mu\text{L/L}$ and the RH was 50 to 60% at the time of the treatment. Plants were watered daily and fertilized with a 20:20:20 (N:P:K) Peters' mix and Peters' micronutrients twice a week, starting at the time of appearance of the first true trifoliolate. Field-grown soybean plants (cv Evans) were planted from seeds in Waukegan silt loam soil (Typic Hapludoll) in St. Paul, MN, during the summer of 1985 in 76-cm rows at 26 plants per meter-row. They were thinned to 13 plants per meter-row 2 weeks later.

Fully expanded soybean trifoliolate leaves at the third or fourth node for the growth chamber-grown plants, and seventh node for the field-grown plants, were used for analysis. Immediately after leaf tissue was harvested, it was frozen either in liquid nitrogen or on dry ice. Samples were promptly stored at -80°C before extraction and assay.

GA_{4/7} were obtained from Abbott Laboratories, and all other chemicals were obtained from Sigma Chemical Co.

Enzyme Extraction

Enzymes were extracted from frozen discs or whole leaves by grinding the tissue with a precooled mortar and pestle using 1:5 (w:v) tissue:buffer with 2% (w/v) polyvinylpyrrolidone. The extract was then homogenized with a Polytron homogenizer (Brinkman Instruments, Westbury, NY) for 20 s, and the tube containing the extract was kept cold with a mixture of acetone and dry ice. In instances in which the homogenizer was not used, 0.5 g of fine glass beads were added to the mortar. All subsequent steps of extraction were done at 0 to 4°C. The grind medium contained 50 mM Hepes-NaOH (pH 7.5), 5 mM MgCl₂, 1 mM EDTA, 2.5 mM DTT, 2 mM GSH, 2% (w/v) PEG-20, and 0.5% (w/v) BSA. The brew was then squeezed through eight layers of cheesecloth. The filtrate was centrifuged at 27,000g for 15 min at 0 to 4°C. The pellet was discarded, and the resulting supernatant, considered the crude extract, was immediately desalted on Sephadex G-25 (Sigma) columns equilibrated with the extraction buffer and then pipetted and used for enzyme assays.

Unless otherwise indicated, all of the tissue samples were conducted at the same time in the morning, approximately 4 h after the onset of light in the growth chamber. This minimized differences in enzyme activity due to diurnal fluctuations that occur in soybeans. Enzyme assays were always performed immediately after extraction.

Enzyme Assays

SPS

SPS was assayed by measuring F-6-P-dependent sucrose (+sucrose-P) formation from UDP-G (17). The assay mixture (70 μL) contained 28 mM UDP-G, 10 mM F-6-P, 15 mM MgCl₂, 10 mM NaF, and 50 mM Hepes-NaOH (pH 7.5). The reactions were initiated by adding an aliquot of leaf extract to the assay mixture. The mixtures were incubated at 30°C for 10 min, and the reactions were terminated by addition of 70 μL of 1 N NaOH. Unreacted fructose and F-6-P were immediately destroyed by immersion of tubes in a boiling water bath for 10 min. The tubes were cooled to room temperature, 250 μL of 0.1% (w/v) resorcinol in 95% ethanol and 750 μL of 30% (v/v) HCl were added, and the mixture was incubated at 80°C for 10 min. After the mixture was cooled to room temperature, the absorbance at 520 nm was determined. The background value was determined by stopping the reaction immediately with the addition of 1 N NaOH, and the standard curve was established using sucrose.

F1,6BPase

Cytoplasmic F1,6BPase was assayed spectrophotometrically by measuring F1,6BP-dependent Pi release under conditions that inhibited chloroplast F1,6BPase activity. The standard reaction medium contained 50 mM Hepes-NaOH (pH 7.6), 5 mM MgCl₂, 10 mM 2-mercaptoethanol, 10 mM NaF, 0.25 mM fructose-1,6-bisphosphate and an aliquot of enzyme extract, all in a final volume of 1 mL. The mixture was incubated for 20 min at 25°C. The activity of nonspecific phosphatase was estimated by omission of MgCl₂ from the reaction mixture, and phosphate production was estimated

by incubating the enzyme extract with the same assay mixture. The reaction was terminated by the addition of 1 mL of 30% (v/v) TCA. Pi was determined by the method described by Rathbun and Betlach (20).

Starch and Sucrose

Starch was assayed by a modification of the methods described by Potter and Breen (19) and Carter et al. (4). Leaf samples (0.7–1 g) that had been stored frozen were boiled with 10 mL of 80% ethanol for 5 min to remove free sugars. The mixture was then homogenized with a mortar and pestle. The homogenate was boiled for 2 min and centrifuged at 12,400g for 5 min. The pellet was collected, and, as necessary, the extraction and centrifugation steps were repeated until the pellet was pigment free. The supernatant fractions were combined, and the pellet was air dried at 60°C overnight. The dried pellet was resuspended in 10 mL of 100 mM acetate buffer (pH 4.8) and boiled for 15 min. The gelatinized starch was digested with 20 mg of amyloglucosidase (grade II, Sigma) for 90 min at 55°C with agitation. Following the digestion, the samples were centrifuged at 12,400g for 5 min. A 50- μ L aliquot of the supernatant was then enzymically assayed for glucose using the glucose oxidase method described by McLaren and Smith (18) and the "Sigma Diagnostics" procedure No. 510. Starch was calculated from glucose content and expressed as milligrams of glucose equivalents per gram fresh weight.

The combined supernatant fractions containing the soluble sugars were evaporated to dryness using a SpeedVac concentrator (Savant, Farmingdale, NY) and utilized to determine sucrose content as described by Potter and Breen (19). Hexose levels in the supernatant fraction were analyzed enzymically using hexokinase, P-glucoisomerase, and glucose-6-P dehydrogenase (6).

ABA

Quantification of endogenous ABA in plant tissue samples was performed as described by Schussler et al. (23). Leaf samples were extracted in 80% methanol and purified by reverse-phase HPLC, and ABA concentration in the samples was quantified using GLC with electron capture detector and appropriate internal standards (23).

RESULTS

Diurnal Pattern of Sucrose-Synthesizing Enzymes

Pronounced diurnal fluctuations were observed in the activity of SPS. The maximum activity occurred at approximately 1600 h, and then SPS activity decreased fairly slowly to reach a low level at about the start of the light period (Fig. 1A). Cytoplasmic F1,6BPase activity in leaf extracts from field plants remained relatively constant throughout the photoperiod (Fig. 2).

Diurnal changes in SPS and F1,6BPase activities under growth chamber conditions were also investigated. Unlike SPS from field-grown plants, the diurnal rhythm of SPS activity in the growth chamber-grown plants presented two peaks (Fig. 1B). It is notable that the pattern of the diurnal

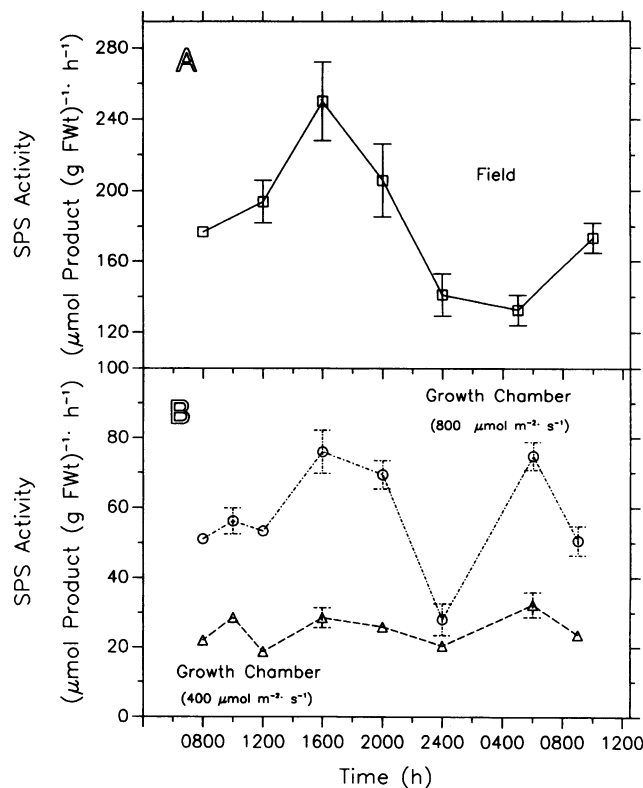


Figure 1. Diurnal variations of SPS activity in soybean leaves. A, Plants grown in the field (□); under these conditions the dark period was from 20:30 to 05:30. B, Plants grown in the growth chamber with irradiance level at 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (○); plants grown and in growth chamber with irradiance level at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the canopy (Δ); under growth chamber conditions the dark period was from 19:00 to 05:00. Field irradiance level reached an optimum of 2700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at approximately midday. Each data point represents the mean value of six samples, and the vertical bars indicate the se. FWt, Fresh weight.

rhythm of SPS activity in plants grown under two different irradiance levels (400 and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$) is similar (Fig. 1B). However, the overall extractable SPS activity was higher when the irradiance level was higher (Fig. 1B), and SPS activity in leaves from growth chamber-grown plants was markedly lower than the activities recovered from field plants (Fig. 1A). Furthermore, F1,6BPase activity from growth chamber-grown plants (800 $\mu\text{mol m}^{-2} \text{s}^{-1}$) exhibited a pattern of variations different from that noted under field conditions with a peak of activity at about 5 h into the light period of the growth chamber-grown plants (Fig. 2). In contrast to SPS, levels of F1,6BPase activity were distinctly higher in leaves from growth chamber-grown plants compared with those from the field.

Recognizing the key role of the enzymes SPS and F1,6BPase in sucrose synthesis, we tested to determine whether there was a correlation between the activities of these two enzymes. Activities of SPS and F1,6BPase in leaf extracts from soybean plants grown in a controlled environment were compared at three sampling times during the first

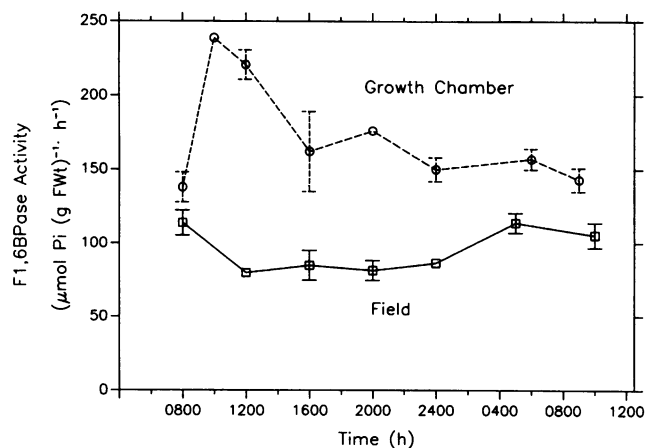


Figure 2. Diurnal changes in the activity of leaf F1,6BPase. Soybean plants were grown in the field (\square) or in the growth chamber at irradiance level of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ (\circ). The dark period was from 20:30 to 05:30 in the field and from 19:00 to 05:00 in the growth chamber. Each data point represents the mean value of six samples, and the vertical bars indicate the SE. FWt, Fresh weight.

part of the light period and 6 h into the dark period. A positive correlation between the activities of the two enzymes was found when studied on a diurnal basis. The correlation coefficients were +0.78 and +0.83 for the light period and the dark period samples, respectively (Table I). Regression analysis of the combined data from light and dark sampling times resulted in a loss of the correlation. This was mainly due to the distinct difference in the slopes (Table I).

Diurnal Changes in Tissue Carbohydrate Levels

Diurnal fluctuations in leaf concentrations of sucrose and starch showed similar patterns in leaves from the field- and the growth chamber-grown plants (Fig. 3). During the first

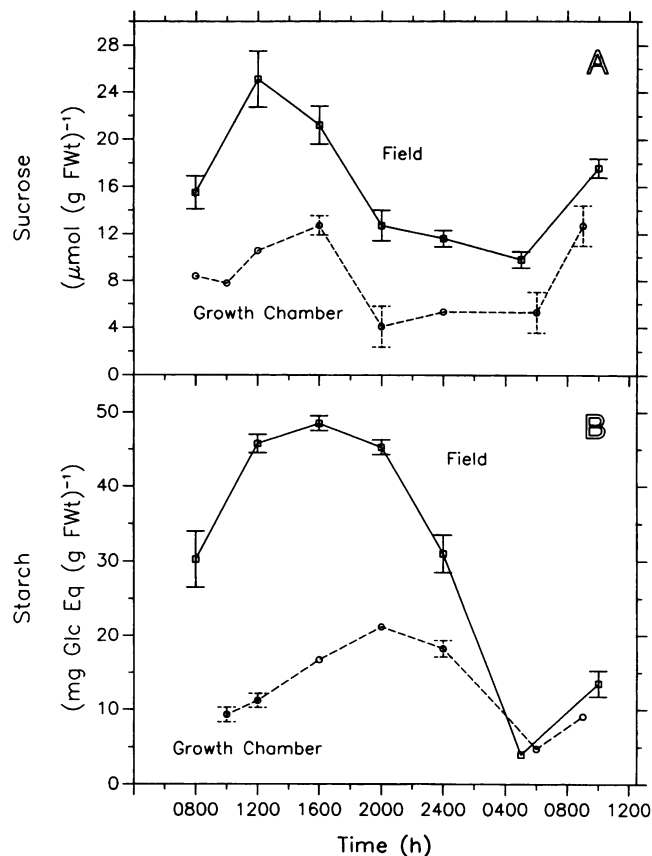


Figure 3. Diurnal fluctuations of nonstructural carbohydrate content in soybean leaves. A, Change in the leaf levels of sucrose were analyzed from field-grown (\square) and growth chamber-grown plants (\circ). B, Variations in starch levels were monitored in the field (\square) and the growth chamber (\circ). The dark period in the field was from 20:30 to 05:30 and from 19:00 to 05:00 in the growth chamber. Each data point represents the mean value of six samples, and the vertical bars indicate the SE. Irradiance level in the growth chamber was measured at $800 \mu\text{mol m}^{-2} \text{s}^{-1}$. FWt, Fresh weight.

Table I. Relationship between Activities of Sucrose-Synthesizing Enzymes and Corresponding Levels of Leaf Nonstructural Carbohydrates and ABA

| Comparison | Regression Parameters | | |
|---|-------------------------|-------|-----------|
| | Correlation coefficient | Slope | Intercept |
| SPS versus F1,6BPase (light samples) | 0.78 ^a | 2.5 | -223 |
| SPS versus F1,6BPase (dark samples) | 0.83 ^a | 0.64 | 132 |
| SPS versus F1,6BPase (light/dark samples) | 0.45 | 0.4 | 126 |
| Sucrose versus F1,6BPase | -0.62 ^b | -0.07 | 22 |
| Sucrose versus SPS | 0.75 ^b | 0.14 | 0.75 |
| Starch versus F1,6BPase | -0.66 ^b | -0.2 | 50 |
| Starch versus SPS | 0.76 ^b | 0.37 | -8.5 |
| F1,6BPase versus ABA | -0.84 ^b | -0.25 | 212 |
| SPS versus ABA | 0.46 | 0.14 | 52 |

^a Statistically significant at $P < 0.05$. ^b Statistically significant at $P < 0.01$.

half of the light period, an accumulation of sucrose was observed under both growing conditions and reached a maximum at 1200 h in leaf tissue from the field and 1600 h in leaves from the growth chamber. The sucrose then declined rapidly thereafter and remained at a constant low concentration through the dark period. Starch accumulated at a much higher rate during the light period, peaked about 4 h after sucrose reached its maximum levels under both conditions, and then declined steadily. By the end of the dark period, most of the accumulated starch was mobilized, and the leaf sucrose pool declined significantly. Leaf starch and sucrose concentrations in field-grown plants were generally 45 to 50% higher than levels obtained in growth chamber-grown plants.

There was no significant diurnal fluctuation of hexose sugar levels (Fig. 4), but hexose levels in the leaves from growth chamber-grown plants were about double the amount in leaves from field-grown plants. This suggests that hexose utilization under field conditions is higher, because starch and sucrose levels were also higher.

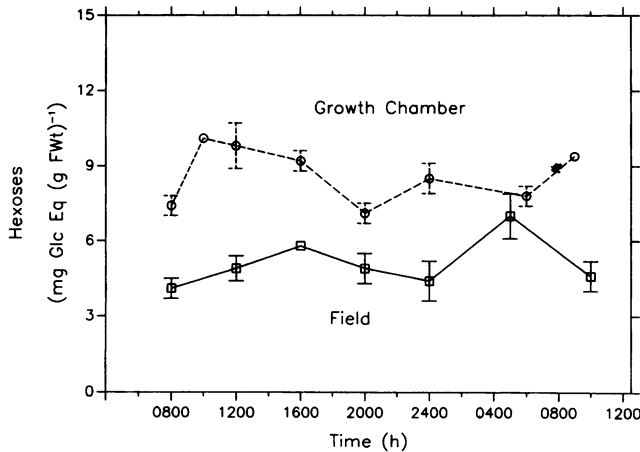


Figure 4. Diurnal changes in soybean leaf hexose levels of field-grown plants (\square , $n = 5$) and growth chamber-grown plants at irradiance level of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ (\circ , $n = 3$). The dark period in the field was from 20:30 to 05:30 and from 19:00 to 05:00 in the growth chamber. The vertical bars indicate the SE. FWt, Fresh weight.

Sucrose and starch levels in soybean leaves were positively correlated with the activity of SPS (Table I), but the regression analysis of the interrelations between F1,6BPase and starch and F1,6BPase and sucrose showed a negative correlation (Table I).

Role of ABA in Leaf Sucrose Synthesis

The diurnal pattern of endogenous ABA content of soybean leaves was investigated in both field- and growth chamber-grown plants. Similar diurnal fluctuations in ABA levels were observed under both growing conditions. However, the ab-

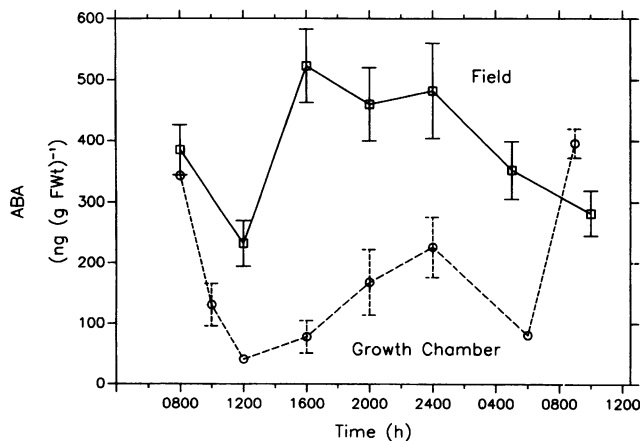


Figure 5. Diurnal variations in leaf ABA content from soybean plants grown in the field (\square) and the growth chamber at irradiance level of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ (\circ). The dark period in the field was from 20:00 to 05:00 and from 19:00 to 05:00 in the growth chamber. Each data point represents the mean value of five samples, and vertical bars indicate the SE. FWt, Fresh weight.

Table II. Effect of Two Cycles of Water Stress on the Extractable Activities of SPS and F1,6BPase, on the Leaf Carbohydrate Content, and on the Endogenous Levels of ABA

Soybean plants were subjected to two periods of drought by withholding water until wilting occurred, followed by 24 h of recovery after rewatering. Leaf samples were collected 2.5 h after illumination commenced in the growth chamber. The irradiance level in the chamber was at $600 \mu\text{mol m}^{-2} \text{s}^{-1}$. Values are the means \pm SE of four plants.

| | Control Plants | Stressed Plants |
|---|----------------|-----------------|
| ABA (ng g^{-1} fresh wt) | 198 ± 34 | 1007 ± 137 |
| SPS activity (μmol of product $\text{dm}^{-2} \text{h}^{-1}$) | 80 ± 8 | 124 ± 8 |
| F1,6BPase activity (μmol of Pi $\text{dm}^{-2} \text{h}^{-1}$) | 480 ± 11 | 318 ± 37 |
| Sucrose ($\mu\text{mol dm}^{-1}$) | 12.9 ± 1.3 | 20.6 ± 3.3 |
| Starch (mg of Glc eq dm^{-1}) | 17.8 ± 1.4 | 30.9 ± 1.8 |
| Leaf water potential (MPa) | -0.7 | -1.8 |

solute levels of ABA were distinctly higher in the field than in the controlled environment (Fig. 5). F1,6BPase activity was negatively correlated with ABA content ($r = -0.84$). However, there was no apparent correlation between SPS activity and ABA content ($r = +0.46$; Table I).

To increase endogenous levels of ABA in the leaf and to study its effect on the extractable activities of SPS and F1,6BPase, growth chamber-grown soybean plants were subjected to two periods of drought stress. After such treatment, leaf endogenous levels of ABA increased 5-fold, F1,6BPase activity decreased, and SPS activity increased nominally (Table II). Exogenous treatment of intact soybean plants with 10^{-6} M ABA decreased the leaf extractable activity of F1,6BPase (25%) but had no significant effect on SPS activity (Table III). Moreover, the plants that were subjected to water deficit had an increase of 37% in leaf sucrose level and 50% in starch content on a fresh weight basis (Table II).

Role of GAs in Carbon Metabolism and Partitioning in the Leaf

When leaves were treated with $\text{GA}_{4/7}$, starch content of this tissue showed a 2-fold increase (Table IV). This enhancement of starch concentration was detectable 2 h after GA application, and the optimum response was observed 24 h following the treatment with 10^{-5} M $\text{GA}_{4/7}$ (Table IV). Furthermore, application of $\text{GA}_{4/7}$ (10^{-6} M to 10^{-5} M) significantly increased leaf sucrose levels, but this increase was observed only at 6 and 24 h posttreatment (Table IV).

To examine the effect of exogenous applications of GAs on the activities of SPS and F1,6BPase in soybean leaves, plants were treated with GA_3 and $\text{GA}_{4/7}$. These treatments significantly increased SPS activity (30–70%) (Table III). GA_3 showed very little effect on the activity of F1,6BPase. However, application of $\text{GA}_{4/7}$ at a concentration of 10^{-5} M resulted in a 97% enhancement in leaf F1,6BPase activity (Table III).

DISCUSSION

The present study revealed a striking difference in carbon metabolism and partitioning within soybean leaves between

Table III. Effect of Exogenous Applications of GAs and ABA on the Activities of Two Key Sucrose-Synthesizing Enzymes

Vegetative soybean plants were treated via capillary wick system with GAs, ABA, or water (control) 24 h before leaf tissue samplings, via capillary wick. Tissue for enzyme analysis was harvested 2.5 h after the illumination commenced in the growth chamber at an irradiance of 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

| | SPS Activity $\mu\text{mol of product dm}^{-2} \text{h}^{-1}$ | F1,6BPase Activity $\mu\text{mol of Pi dm}^{-2} \text{h}^{-1}$ |
|--------------------------------------|--|---|
| Control ^a | | |
| Third leaf ^d | 143 ± 38 | ND ^e |
| Fourth leaf | 167 ± 33 | 165 ± 19 |
| 10 ⁻⁶ M GA ₃ | | |
| Third leaf | 227 ± 37 | ND |
| Fourth leaf | 283 ± 100 | 137 ± 19 |
| Control ^b | 148 ± 5 | 120 ± 13 |
| 10 ⁻⁵ M GA _{4/7} | 193 ± 7 | 237 ± 28 |
| Control ^c | 187 ± 9 | 258 ± 26 |
| 10 ⁻⁶ M ABA | 178 ± 6 | 193 ± 8 |

^a Values represent the means ± SE of six replicates. ^b Four replicates were used per treatment. ^c Values represent the means ± SE of eight replicates. ^d The leaf number corresponds to its node position with regard to the cotyledonary leaves. ^e ND, Not determined.

plants grown in the field and those grown under a controlled environment. Diurnal fluctuations of leaf SPS activity from field-grown soybean plants (Fig. 1A) demonstrated a pattern similar to that reported earlier by Rufty et al. (21) and Huber et al. (13) in greenhouse-grown soybean plants. Additional studies of diurnal variations of SPS activity have also been reported to vary among different plant species, including sugar beet, tobacco, peas, spinach, cotton, and maize (16, 27). The results obtained here show that the diurnal rhythm in leaf soybean SPS activity was different in plants grown under the two environmental conditions tested (namely, field versus growth chamber). The present data support earlier reports that indicated that diurnal fluctuation in SPS activity in soybean leaves is a mechanism controlled by an endogenous clock (21) and that soybeans are among species that do not exhibit light activation of SPS (14). This is because of the consistency of the pattern of diurnal fluctuations and the fact that the measurable activity of SPS was still detected at a high level during the first hours of darkness. The alterations in extractable enzyme activity in response to changes in the light could simply be a measurement of a light effect on photosynthetic activity and levels of substrates in the leaf (3).

In leaves from field-grown plants, cytosolic F1,6BPase showed a certain diurnal stability during the daily light/dark cycle (Fig. 2). This diurnal stability was reported in greenhouse-grown soybean plants (21), but in growth chamber-grown plants, F1,6BPase exhibited a sharp increase in detectable activity at about the middle of the light period. The fact that this peak of activity coincides with a distinct decrease in ABA leaf content (Fig. 5), that exogenous ABA application and water stress (Tables II and III) both decreased F1,6BPase activity, and that the regression analysis of F1,6BPase activity

and leaf ABA levels revealed a negative correlation (Table I) suggests that the fluctuations in the activity of this enzyme observed in the growth chamber may be due directly or indirectly to changes in leaf ABA levels in lieu of a direct control by an endogenous clock. Additionally, it was observed that ABA levels in the field were 4 to 5 times greater than those observed in the growth chamber and that the extractable F1,6BPase activity was distinctly lower in the field. This correlation between F1,6BPase activity and ABA levels appears to be more apparent during the light period than during the dark period.

The pattern of diurnal changes of sucrose and starch levels was similar under both field and controlled-environment conditions, but those levels were generally twice as high in the field-grown plants as in the growth chamber-grown plants. Moreover, both starch and sucrose accumulated in soybean leaves during the light period, and then their levels were rapidly depleted by the end of the dark period. The fact that starch levels always peaked about 4 h following the highest sucrose levels supports earlier reports that soluble carbohydrates are believed to be mobilized before the insoluble carbohydrates are degraded (21, 25).

It is difficult to reach conclusions from our study about the pattern of sucrose compartmentation in the leaf, which in turn makes it relatively complex to study correlations of leaf sucrose content and activities of sucrose-synthesizing enzymes. Nevertheless, it has been suggested that SPS activity reflects the capacity of the sucrose biosynthetic system to develop in the light (8). When regression analysis was conducted between leaf endogenous sucrose levels and SPS activity on a diurnal basis, a positive correlation was revealed. On the other hand, sucrose content and activities of F1,6BPase presented a negative correlation. This could possibly be due to the lack of consistent diurnal fluctuations in

Table IV. Effect of a GA Spray Application on Leaf Tissue Content in Starch and Sucrose: Concentration and Time-Course Study

The data represent the means ± SE of five replicates. All tissue samplings occurred 2 h after illumination started.

| | Starch $\text{mg of Glc eq dm}^{-2}$ | Sucrose $\mu\text{mol dm}^{-2}$ |
|--|---|------------------------------------|
| Experiment I ^a | | |
| Control | 19.5 ± 1.5 | 28.6 ± 6.3 |
| + GA _{4/7} | | |
| 10 ⁻⁶ M | 30.7 ± 5.8 | 47.6 ± 12.0 |
| 10 ⁻⁵ M | 39.8 ± 10.0 | 33.6 ± 5.3 |
| 10 ⁻⁴ M | 39.4 ± 9.4 | 37.1 ± 3.8 |
| Experiment II ^b | | |
| Control | 4.9 ± 2.0 | 3.2 ± 0.3 |
| + GA _{4/7} (10 ⁻⁵ M) | | |
| 2 h | 14.0 ± 1.5 | 1.3 ± 0.0 |
| 6 h | 15.3 ± 2.4 | 7.3 ± 2.7 |
| 24 h | 17.9 ± 4.7 | 6.2 ± 2.4 |

^a Experiment I, Irradiance level in the chamber was 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Treatment time with GA_{4/7} or water was 24 h.

^b Experiment II, Irradiance level in the chamber was 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Treatments with GA_{4/7} or water (control) were made at different times so that all tissue samplings occurred at the same time.

F1,6BPase activity to match those of SPS and sucrose. Additionally, the high activities of F1,6BPase found in the first part of the photoperiod usually correspond to the interval of high sucrose export rate (12).

It has been reported that starch synthesis does not seem to reflect fixation of excess carbon; rather, it may represent a preprogrammed portion of the total carbon fixed (27). This signifies the existence of competition between starch and sucrose for available carbon and also their close relation to the carbon assimilation rate. These two aspects of starch metabolism could explain the positive correlation observed in the present study between leaf starch content and SPS activity, because a decrease in starch degradation or an increase in starch synthesis both lead to an augmentation in starch levels. Furthermore, it was reported by Huber (10) that soybeans presented both high absolute levels of starch and high SPS activity and that normalizing the starch data with photosynthetic rate resulted in a negative correlation between starch content and SPS activity. Nevertheless, our results provide evidence for a significant negative correlation between F1,6BPase and starch levels in the leaf (Table I).

The results from the present study indicate that GAs play an important role in carbohydrate partitioning in the leaf. One of the major effects seems to be on SPS activity on which this GA effect appears to be very fast (2 h after application [6]). Similar GA effects on leaf disc SPS activity in sugar beet and bean leaves have been reported (7). It is very significant that SPS distinctly responds to application of GAs. However, it remains unclear how GAs enhance the activity of the enzyme, whether by activation of the already existing enzyme or by increase in the synthesis of new protein. This aspect of GA action is the subject of a follow-up paper (6).

Elevated levels of GAs appear to consistently increase starch and sucrose levels in soybean leaves. The same response has been reported in citrus leaves, in which GA₃ increased levels of soluble sugars and starch and decreased activities of leaf α - and β -amylases (22). Hence, the GA-enhanced accumulation of starch observed in our investiga-

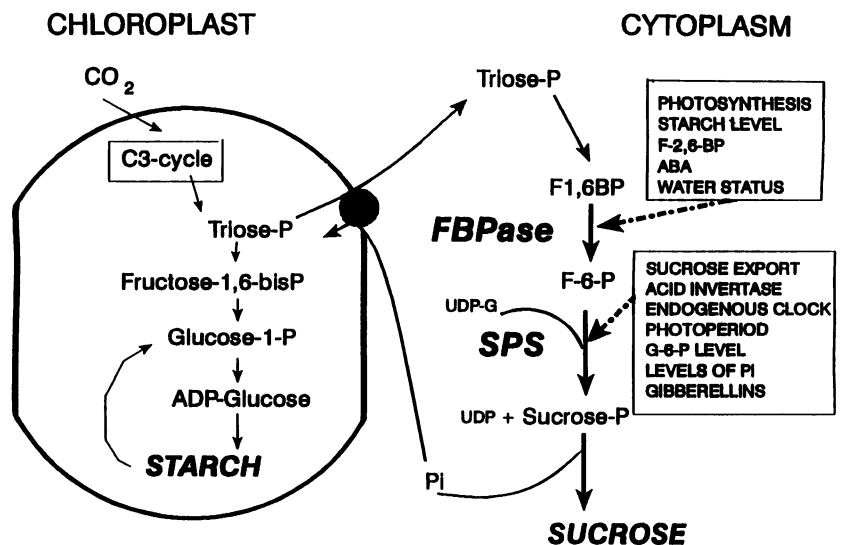
tion could simply be due to a decrease in its degradation. It is important to note that the carbon assimilation rate remained unchanged in response to GA treatment (data not shown [6]). The decrease in sucrose levels 2 h following GA treatment could be explained in two ways: the simultaneous enhancement of acid invertase activity 2 h following GA application (6) or the increase in sucrose export from the leaf (1, 5).

It is apparent from our study that F1,6BPase activity correlates negatively with tissue levels of ABA and starch. And it is well documented that the substrate F2,6BP is a potent inhibitor of cytosolic F1,6BPase and leads to increased starch synthesis (26). There are also indications that F2,6BP levels increase when leaves are subjected to water stress. It is not clear, however, whether F2,6BP is altered as a primary or secondary factor in stressed leaves (15, 27). The direct control mechanism involved between F2,6BP and F1,6BPase remains to be investigated, but in the present study there is strong evidence that both water stress and ABA decrease the activity of F1,6BPase. Whether this response is due to changes in photosynthesis, levels of F2,6BP and starch, or direct effect on F1,6BPase is a topic for future studies.

CONCLUSIONS

The present study demonstrates the considerable influence of environmental factors on carbon metabolism and partitioning in soybean leaves. It is apparent that plants behave differently when grown in controlled as compared with field environments, where the major differences are temperature, water status, light intensity, and light quality. Plants in the field seem to have higher levels of ABA, starch, and sucrose and also greater SPS activity. However, plants in the growth chamber exhibit higher hexose content and increased F1,6BPase activity. The diurnal changes observed in the activities of SPS and F1,6BPase under the two growing environments seem to be attributed to some of the key biochemical factors that are involved in the timing of assimilate availability in leaves. In addition, these diurnal varia-

Figure 6. Simplified illustration of the possible levels of control along the biosynthetic pathway of the photosynthetic sucrose in leaves. G-6-P, glucose-6-phosphate; FBPase, fructose-1,6-bisphosphatase.



tions in enzyme activity appear to be closely aligned with changes in leaf carbohydrate levels under both growing conditions.

The plant growth substances, ABA and the GAs, also seem to affect carbohydrate partitioning within source leaves, which may be another important control mechanism. However, the mode of action of these plant hormones in this process requires more studies.

Although F1,6BPase and SPS appear to be key points in the regulation of sucrose synthesis, it is important to emphasize that this regulation is the property of an entire pathway rather than individual processes. Therefore, an investigation of interactions and coordination between these enzymes seems to be of great importance. The absence of a significant positive correlation between SPS and F1,6BPase enzymes suggests that the factors that regulate them may be different. There is a possibility that each enzyme represents a separate control point along the metabolic pathway of sucrose synthesis. If this is the case, the question of which step in the pathway is the key step in sucrose synthesis remains unanswered.

One proposed hypothesis is that there are two key apparent control points along the pathway of sucrose synthesis (Fig. 6). The first level of regulation is at that of F1,6BPase, where generally the regulation of the activity of this enzyme appears to be closely correlated with events occurring at the beginning level of carbon metabolism in the leaf, such as photosynthetic activity, changes in starch levels in the chloroplast, and response to variations in tissue levels of ABA and/or F2,6BP. The second level of regulation is that of SPS, in which the activity of this enzyme was observed to display a stronger relationship with parameters at the other end of the carbohydrate metabolism pathway, such as sucrose export, acid invertase activity, the endogenous clock controlling diurnal fluctuations in substrate levels and enzyme activities, changes in the photoperiod, change in levels of sucrose, glucose-6-phosphate and Pi, and response to GAs.

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