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Role of Sucrose-Phosphate Synthase in Partitioning of Carbon in Leaves[1]

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STEVEN C. HUBER
United States Department of Agriculture and Departments of Botany and Crop Science, North Carolina State University, Raleigh, North Carolina 27650

ABSTRACT

Variations in leaf starch accumulation were observed among four species (wheat [*Triticum aestivum* L.], soybean [*Glycine max* L. Merr.], tobacco [*Nicotiana tabacum* L.], and red beet [*Beta vulgaris* L.]), nine peanut (*Arachis hypogea* L.) cultivars, and two specific peanut genotypes grown under different nutritional regimes. Among the genotypes tested, the activity of sucrose phosphate synthase was correlated negatively with leaf sucrose content in seven of the nine peanut cultivars as well as the two peanut cultivars grown with different mineral nutrition. The peanut cultivars differed in the effect of 10 millimolar sucrose on sucrose phosphate synthase activity in leaf extracts. Enzyme activity in crude leaf extracts was inhibited by sucrose (10–42%) in four of the cultivars tested whereas five cultivars were not. Overall, the results suggest that a correlation exists between the activity of sucrose phosphate synthase and starch/sucrose levels in leaves.

There is considerable interest in the parameters that control the partitioning of photosynthetically fixed carbon between starch and sucrose. In leaves, starch is an insoluble end product which fluctuates on a diurnal basis, whereas leaf sucrose content reflects the balance between formation and export. Provision of leaf sucrose is one important factor that controls the rate of translocation from source leaves (3). Further, recent evidence suggests that carbon partitioned into starch is utilized primarily for growth of shoots (stems and expanding leaves), as opposed to roots, in the dark (1; and Huber, unpublished). Consequently, carbon partitioning in leaves may influence allocation of carbon and growth activities in the whole plant.

It has been postulated that the rate of sucrose formation may control the rate of starch formation (7–10) by affecting the cytoplasmic concentration of Pi and P-esters. Rapid sucrose formation in the cytosol would result in elevated Pi levels, which would rapidly exchange with metabolic intermediates such as triose-P in the chloroplast. As a result, carbon would be diverted from starch formation (7, 16). In the sucrose formation pathway, the activities of cytoplasmic fructose-1,6-bisphosphatase and SPS[2] are low relative to other enzymes (6, 9), and thus, are likely points of regulation. The activity of SPS in leaf extracts appears to be just sufficient to account for photosynthetic sucrose formation *in situ*. Furthermore, variation among species has been observed in the activity of SPS in leaf extracts, and it was noted that leaf starch content was correlated negatively with SPS activity (9).

The specific objectives of the present study were to relate SPS activity in leaf extracts with leaf starch accumulation and leaf sucrose content, using comparisons among species and cultivars, as well as specific genotypes exposed to a variety of nutritional regimes.

MATERIALS AND METHODS

Three separate experiments were conducted with several genotypes to examine the role of SPS in carbon partitioning in leaves.

Plant Growth and Harvests. Wheat (*Triticum aestivum* L. cv Butte), soybeans (*Glycine max* L. Merr. cv Arksoy), tobacco (*Nicotiana tabacum* L. cv Coker 139), and red beets (*Beta vulgaris* L. cv Detroit Dark Red) were grown in sand in a greenhouse. Plants were watered as necessary with tap water in the morning. In the afternoon, all pots received approximately 600 ml of standard nutrient solution of the following composition: NO_3^- (7.5 mM); $H_2PO_4^-$ (0.5 mM); K^+ (3 mM); Ca^{2+} (2.5 mM); Mg^{2+} (1 mM); and SO_4^{2-} (1 mM). Trace element composition was: H_3BO_4 (46 μM); $MnCl_2$ (9.1 μM); $ZnSO_4$ (1.9 μM); $CuSO_4$ (0.3 μM); Na_2MoO_4 (0.52 μM); and (Fe) Sequestrene (0.02% w/v). Eight plants of each of the four species were harvested for plant growth analysis at 23 and 27 d after sowing. Leaf starch accumulation was measured and samples taken for enzyme analysis as described below.

Nine peanut (*Arachis hypogea* L.) cultivars were grown in soil in a greenhouse to identify intraspecific variation. After 55 d of growth, two replicate pots (one plant/pot) were analyzed for starch accumulation and samples were taken for enzyme analysis. In a separate study, two peanut cultivars ('NC-6' and '350680') were grown under a variety of nutritional conditions. The treatments were as follows: (a) control (standard nutrient solution: 3 mM K^+, nil Na^+, 0.5 mM $H_2PO_4^-$); (b) low K^+ (0.5 mM); (c) high K^+ (10.5 mM); (d) low K^+ (0.5 mM), high Na^+ (10 mM); and (e) high phosphate (1.25 mM). The concentrations of K^+ and Na^+ were varied, as indicated, as the sulfate salts. Each treatment consisted of five replicate pots (one plant/pot), and plants were harvested 4 weeks after the treatments began.

In each experiment, plant harvests were used to calculate net carbon assimilation per g dry weight of leaves (NCA) and relative assimilation rates (RAR), which were used as estimates of leaf photosynthetic activity.

In the peanut nutrition experiments, direct measurements of CER were obtained with a Beckman Differential IR gas analyzer in an open gas system.[3] Attached leaves were enclosed by a clamp-

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[2] Abbreviations: SPS, sucrose-phosphate synthase; NCA, net carbon assimilation (g dry matter/g leaf); RAR, relative assimilation rate (g dry matter/g leaf·d^{-1}); CER, carbon exchange rate (mg CO_2/g leaf·h^{-1}).

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

on Plexiglas leaf chamber (10 cm^2). Compressed air (355 $\mu l/l$ CO_2) was passed into the leaf chamber at a rate of 1.5 l/min.

Starch Analysis. Samples for leaf starch were usually collected at 0800 and 1700 h. The difference between afternoon and morning starch contents is referred to as leaf starch accumulation, and is expressed on a leaf area or weight basis. Leaf samples were homogenized in 80% ethanol, and starch was estimated as glucose released by amyloglucosidase treatment of the ethanol-insoluble fraction of leaf samples as previously described (10). Typically, separate samples were taken from the most recent fully expanded leaves of two to three plants. Results presented represent mean values for starch accumulation.

Leaf Sugar and Enzyme Assays. After taking the afternoon starch sample, the remaining leaf tissue was frozen at −80°C. Samples were extracted (10) and immediately thereafter, an aliquot was diluted 5-fold with water and placed in a boiling water bath for 2 min. Sucrose and hexose contents were measured enzymically (11) on the cooled supernatants. The remaining sample was assayed for SPS activity within 1 h after extraction. Sucrose-P synthase was assayed by measurement of fructose-6-P-dependent formation of sucrose (+sucrose-P) from UDP-glucose. The assay mixture (70 μl) contained 8 mM UDP-glucose, 8 mM fructose-6-P, 15 mM $MgCl_2$, 40 mM Hepes-NaOH (pH 7.5), and desalted leaf extract. Mixtures were incubated at 25°C for 10 min, and reactions were terminated by addition of 70 μl N NaOH. Unreacted fructose-6-P was destroyed by placing the tubes in boiling water for 10 min. After cooling, 0.25 ml of 1% resorcinol in ethanol and 0.75 ml of 30% HCl was added, and the tubes were incubated at 80°C for 8 min (14). The tubes were chilled on ice and centrifuged at 1,500g for 5 min, and the A_{520} was measured. In experiments with peanut leaf extracts, SPS assays were conducted with and without 10 mM sucrose, and production of UDP was quantitated following the procedure of Salerno and Pontis (15).

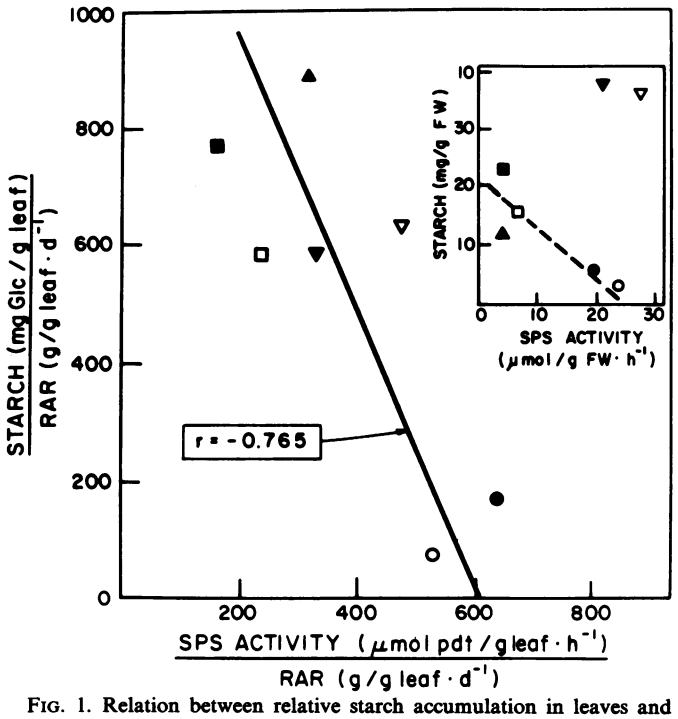

FIG. 1. Relation between relative starch accumulation in leaves and SPS activity in leaf extracts of wheat (○, ●), soybean (▽, ▼), tobacco (□, ■), and red beet (▲) seedlings at 23 d (○, ▽, □) and 27 d (●, ▼, ■, ▲). RAR was used as an estimate of leaf photosynthetic activity. The inset shows absolute values of leaf starch and SPS activities.

RESULTS

Variation among Species and Cultivars. Seedlings of wheat, soybeans, tobacco, and red beet were compared at two sampling dates for leaf starch accumulation and the activity of SPS in leaf extracts. In general, wheat leaves accumulated the lowest amounts of starch, expressed on either a fresh weight or dry weight basis, and also had the highest activities of SPS in leaf extracts. Tobacco and red beet leaves accumulated high levels of starch and had lower activities of SPS.

The four species differed in leaf photosynthetic activity, estimated as relative assimilation rates (dry weight/g dry weight leaf · d^{-1}) from plant harvest data (data not shown). The ratio of leaf starch accumulation to RAR, hereafter referred to as relative leaf starch accumulation, provided an estimate of the proportion of photosynthetically fixed carbon that was partitioned into starch. Relative leaf starch accumulation and SPS activity (normalized with RAR) in leaves of the four species at two sampling dates are compared in Figure 1. As shown, starch accumulation was correlated negatively ($r = -0.765$) with SPS activity. The inset shows the same data, but without normalization with RAR values. With wheat, tobacco, and red beet, absolute levels of leaf starch tended to be correlated negatively with absolute SPS activities. However, soybean leaves had both high levels of starch and SPS activity, and hence, did not conform to the same pattern as the other species. Because soybean had the highest RAR of the four species, normalizing the data with photosynthetic rate resulted in a correlation that held for all four species (Fig. 1).

Variation in leaf starch accumulation and activity of SPS in leaf extracts was also observed among peanut cultivars. In general, all of the peanut cultivars tended to accumulate substantial levels of leaf starch and to have relatively low activities of SPS. Interestingly, the cultivars differed in the effects of sucrose on SPS activity. Sucrose (10 mM) inhibited SPS activity (10–42%) in leaf extracts prepared from four of the cultivars, and stimulated activity slightly in extracts from five of the cultivars (data not shown). As before, leaf starch accumulations and SPS activities were normalized with an estimate of leaf photosynthetic activity (NCA from plant harvest data, not shown) for comparisons among the cultivars. Figure 2A compares relative leaf starch accumulation with SPS activity (assayed in the presence of 10 mM sucrose) in leaf extracts of the nine cultivars. As shown, starch accumulation was correlated negatively ($r = -0.63$) with SPS activity.

The peanut cultivars tested also differed in leaf sucrose content. With the exception of two cultivars (designated 6 and 7), leaf sucrose content was correlated positively ($r = +0.69$) with activity of SPS in leaf extracts (Fig. 2B).

Effect of Plant Nutrition. Two peanut cultivars (NC-6 and 350680) were grown under a variety of nutritional regimes in order to determine whether a range of nutritionally induced differences in leaf starch formation (of a given genotype) were related to changes in SPS activity in leaf extracts. Peanut cv NC-6 and cv 350680 had generally similar CER (about 40 mg $CO_2/dm^2 \cdot h^{-1}$) when grown under a variety of conditions. Across the nutritional treatments, NC-6 tended to accumulate more leaf starch and maintain a lower leaf sucrose content compared to cv 350680. Using all of the data obtained with both genotypes, a negative correlation was obtained between relative leaf starch accumulation and activity of SPS (assayed in the presence of 10 mM sucrose) ($r = -0.79$; Fig. 3A). In contrast, leaf sucrose content was correlated positively with the activity of SPS in leaf extracts ($r = 0.78$; Fig. 3B). Similar correlations were obtained when the activity of SPS in the absence of sucrose was used (data not shown).

DISCUSSION

In general, the activity of SPS in leaf extracts was correlated negatively with leaf starch accumulation (Figs. 1, 2A, and 3A) but

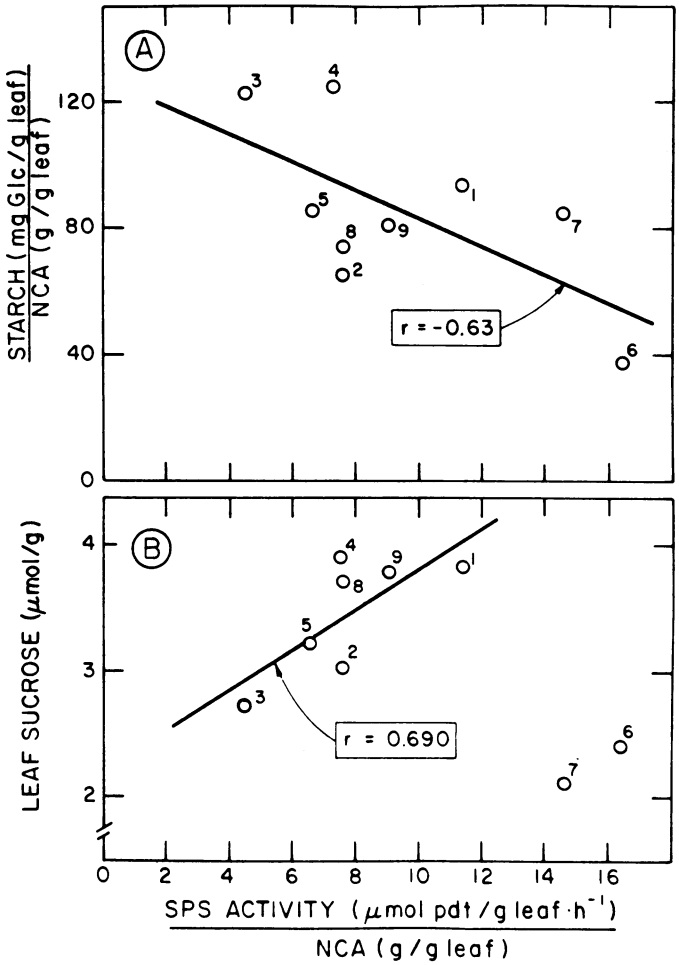

FIG. 2. Relation between (A) leaf starch accumulation or (B) leaf sucrose content and activity of SPS (assayed with 10 mM sucrose) in leaf extracts of nine peanut cultivars. NCA, calculated from plant harvest data, was used as an estimate of leaf photosynthetic activity. Cultivars: 1, '275751'; 2, '275744'; 3, '259747'; 4, '262090'; 5, '275735'; 6, '350680'; 7, Florigiant; 8, Tennessee Red; 9, 'NC-6'. Leaf starch accumulation ranged from 16 (cv 6) to 63 (cv 4) mg Glc/g fresh weight.

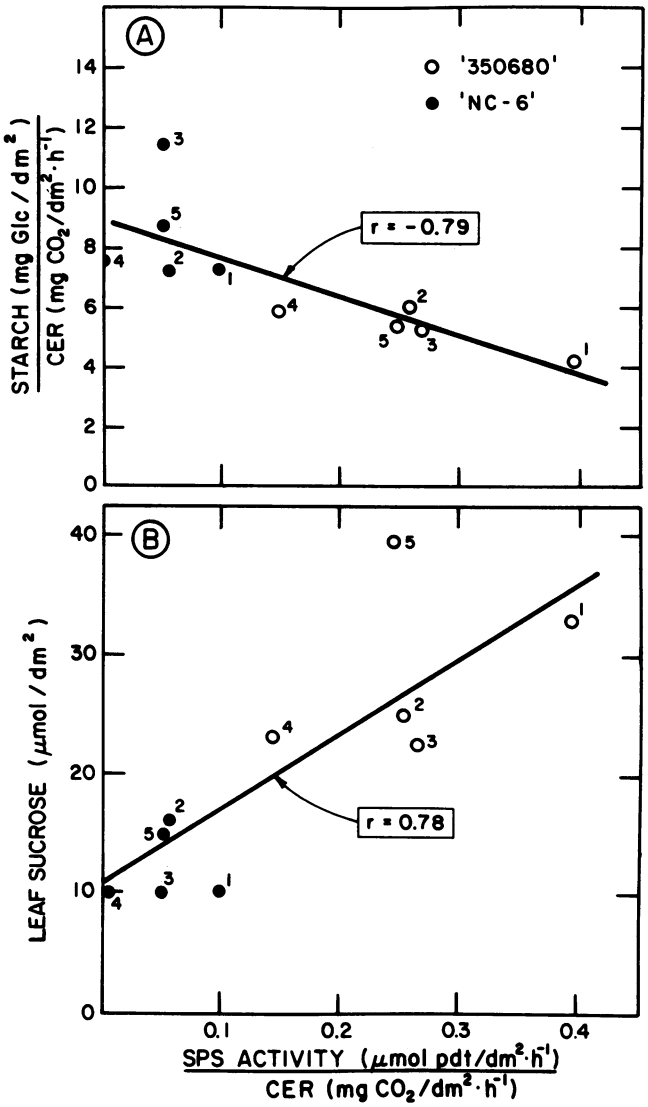

FIG. 3. Relation between (A) leaf starch accumulation or (B) leaf sucrose content and activity of SPS (assayed with 10 mM sucrose) in leaf extracts of two peanut cultivars grown under different nutritional conditions. The numbers refer to treatments that are identified in "Materials and Methods."

positively with leaf sucrose content (Figs. 2B and 3B), regardless of whether variation was related to genotype or plant nutrition. These relationships required that measurements be made at the same leaf position (*e.g.* most recent fully expanded leaf), in combination with an estimate of leaf photosynthetic activity. In some cases (*e.g.* peanut cultivars), correlations were improved when SPS activity was measured in the presence of a low concentration of sucrose (10 mM). In crude leaf extracts prepared from different species, sucrose was found to either stimulate slightly, have no effect, or inhibit activity of SPS (9). It is apparent that such differences in the response of SPS to sucrose may also exist among peanut cultivars. However, detailed kinetic studies with the purified enzyme will be required in order to understand the possible control of SPS activity by sucrose.

The activity of SPS in leaf extracts appears to be just sufficient to account for photosynthetic sucrose formation, and may be one of the key control points in the pathway (6). Starch and sucrose act as competitive end products for fixed carbon because increased formation of one occurs at the expense of the other (8, 10). Control of starch formation by the rate of sucrose biosynthesis can be rationalized on the basis of Pi recycling in the cytoplasm (7, 16).

Translocation studies have identified source leaf sucrose content as an important factor in controlling translocation velocity (3, 4). Leaf sucrose can be compartmented in multiple pools (12), some of which may function as storage pools. However, under steady-state conditions, the transport pool appears to be in equilibrium with the total leaf sucrose pool (2). Consequently, changes in the rate of photosynthetic sucrose formation would be reflected in total leaf sucrose content. In the present study, activity of SPS in leaf extracts was correlated positively with leaf sucrose content among closely related genotypes and within two genotypes grown under various nutritional regimes. The results support earlier proposals (3, 5, 13) that sucrose synthesized by SPS reflects the sucrose pool available for transport.

Carbon partitioning between starch and sucrose appears to be genetically controlled (8, 10), but can be modified by environmental factors such as photoperiod (1) and plant nutrition (3). One factor that may be related to the control of carbon partitioning appears to be the activity of SPS.

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LITERATURE CITED

1. CHATTERTON NJ, JE SILVIUS 1979 Photosynthate partitioning into starch in soybean leaves. I. Effects of photoperiod *versus* photosynthetic period duration. Plant Physiol 64: 749–753
2. CHRISTY AL, CA SWANSON 1976 Control of translocation by photosynthesis and carbohydrate concentrations of the source leaf. *In* IF Wardlaw, JB Passioura, eds, Transport and Transfer Processes in Plants. Academic Press, New York, pp 329–338
3. GEIGER DR 1979 Control of partitioning and export of carbon in leaves of higher plants. Bot Gaz 140: 241–248
4. GEIGER DR, JW BATEY 1967 Translocation of ^{14}C sucrose in sugar beet during darkness. Plant Physiol 42: 1743–1749
5. GIAQUINTA R 1978 Source and sink leaf metabolism in relation to phloem translocation. Carbon partitioning and enzymology. Plant Physiol 61: 380–385
6. HARBRON S, CH FOYER, DA WALKER 1981 The purification and properties of sucrose-phosphate synthetase from spinach leaves: the involvement of this enzyme and fructose bisphosphatase in the regulation of sucrose biosynthesis. Arch Biochem Biophys 212: 237–246
7. HEROLD A 1980 Regulation of photosynthesis by sink activity—the missing link. New Phytol 86: 131–144
8. HUBER SC 1981 Inter- and intra-specific variation in photosynthetic formation of starch and sucrose. Z Pflanzenphysiol 101: 49–54
9. HUBER SC 1981 Interspecific variation in activity and regulation of leaf sucrose phosphate synthase. Z Pflanzenphysiol 102: 443–450
10. HUBER SC, DW ISRAEL 1982 Biochemical basis for partitioning of photosynthetically fixed carbon between starch and sucrose in soybean (*Glycine max* Merr.) leaves. Plant Physiol 69: 691–696
11. JONES MGK, WH OUTLAW, OH LOWRY 1977 Enzymic assay of 10^{-7} to 10^{-14} moles of sucrose in plant tissue. Plant Physiol 60: 379–383
12. OUTLAW WH, DB FISHER, AL CHRISTY 1975 Compartmentation in *Vicia faba* leaves. II. Kinetics of ^{14}C-sucrose redistribution among individual tissues following pulse labeling. Plant Physiol 55: 704–711
13. POLLOCK CJ 1976 Changes in the activity of sucrose-synthesizing enzymes in developing leaves of *Lolium temulentum*. Plant Sci Lett 7: 27–31
14. ROE JH 1934 A colorimetric method for the determination of fructose in blood and urine. J Biol Chem 107: 15–22
15. SALERNO GL, HG PONTIS 1978 Studies on sucrose phosphate synthetase: the inhibitory action of sucrose. FEBS Lett 86: 263–267
16. WALKER DA, A HEROLD 1977 Can the chloroplast support photosynthesis unaided? Plant Cell Physiol Special Issue: 295–310