Plant Physiol. (1989) 89, 225–230
0032-0889/89/89/0225/06/$01.00/0

Received for publication May 10, 1988
and in revised form August 16, 1988

Influence of Phosphorus Nutrition on Growth and Carbon Partitioning in *Glycine max*

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ABSTRACT

Soybean plants (*Glycine max* [L.] Merr var Amsoy 71) were grown in growth chambers with high-phosphorus (high-P) and low-phosphorus (low-P) culture solutions. Low-P treatment reduced shoot growth significantly 7 days after treatment began. Root growth was much less affected by low-P, there being no significant reduction in root growth rate until 17 days had elapsed. The results suggest that low-P treatment decreased soybean growth primarily through an effect on the expansion of the leaf surface which was diminished by 85%, the main effect of low-P being on the rate of expansion of individual leaves. Low-P had a lesser effect on photosynthesis; light saturated photosynthetic rates at ambient and saturating CO_2 levels were lowered by 55 and 45%, respectively, after 19 days of low-P treatment. Low-P treatment increased starch concentrations in mature leaves, expanding leaves and fibrous roots; sucrose concentrations, however, were reduced by low-P in leaves and increased in roots. Foliar F-2,6-BP levels were not affected by P treatment in the light but in darkness they increased with high-P and decreased with low-P. The increase in the starch/sucrose ratio in low-P leaves was correlated primarily with changes in the total activities of enzymes of starch and sucrose metabolism.

Suboptimal phosphorus supply diminishes photosynthetic CO_2-fixation rates (2, 24) and the expansion of the photosynthetic leaf surface (5,22). It may also lead to decreased cytosolic orthophosphate levels (26). Orthophosphate (Pi) is thought to regulate the activities of several enzymes involved in starch and sucrose metabolism *in vivo* and the export of C out of the chloroplast via the Pi-translocator (3). However, much of the evidence in support of Pi as a key regulator of carbon partitioning has been obtained with *in vitro* systems. In this paper, we study the nutritional effects of Pi on growth, photosynthesis, and starch/sucrose metabolism in an intact plant system; soybean plants were treated with sufficient P for optimal plant growth (high-P) and with suboptimal P levels (low-P).

METHODS AND MATERIALS

Plant Culture

Soybean seeds (*Glycine max* [L.] Merr var Amsoy 71) were germinated for 4 d in moistened vermiculite and transferred to 20 L buckets, three plants per bucket, of either high-phosphorus (high-P) or low-phosphorus (low-P) aerated nutrient solution for a maximum of 21 d. All high-P and low-P plants were grown in a Missimer growth chamber with a 16 h light period at 29°C and an 8 h dark period at 23°C. The light intensity was maintained at 525 ± 25 μmol quanta·$m^{-2}\cdot s^{-1}$.

The high-P and low-P solutions were similar except that the high-P solution contained 200 μM KH_2PO_4 and the low-P solution contained 10 μM KH_2PO_4. Both high-P and low-P culture solutions contained 9.125 mM NO_3-N, 0.625 mM NH_4^+-N, 4 mM K^+, 1.0 mM S, 2.5 mM Ca^{2+}, 1.0 mM Mg^{2+}, 250 μM Na^+, 250 μM Si, 500 μM Cl^-, 50 μM FeHEDTA, 25 μM B, 1.0 μM Mn, 1.0 μM Zn, 0.4 μM Mo, and 0.4 μM Cu. The presence of Si in combination with a 10-fold reduction in the Mn concentration in solution ameliorated a putative Mn toxicity symptom in both high-P and low-P plants.

Gas Exchange

Gas exchange analyses were performed on randomly selected high-P and low-P plants at 18 to 20 d after transplant. Fully expanded trifoliates at the second or third node numbered basipetally from the first primary trifoliate were used for both irradiance and CO_2 saturation curve determinations using steady state gas exchange equipment described previously (25). CO_2 concentrations of 305 to 315 $\mu L\cdot L^{-1}$ were used for the irradiance saturation curves and 1500 $\mu L\cdot L^{-1}$ CO_2 concentration to obtain the CO_2-saturated photosynthetic rates.

Leaf area measurements were made using a Decagon leaf area meter (Delta-T model).

Starch and Sucrose Determinations

Leaf punches (3·1.48 cm^2) or fresh fibrous root samples were taken from high-P and low-P plants and frozen in liquid N_2. Leaf punches were frozen in the light or dark depending on the sampling time. Root fresh weights were measured before freezing in liquid N_2. Replicate samples were generally taken from different plants. Samples were ground immediately in 80% ethanol and incubated at 40°C for 18 h and subsequently centrifuged for 10 min at 9000*g* (6). The supernatant was evaporated to dryness and redissolved in 2 mL of H_2O and 0.5 mL of chloroform. Chl-containing chloroform was removed by spinning at 3000*g* for 10 min and the clear supernatant retained for glucose and sucrose analysis. Sucrose was converted to glucose and fructose with an invertase (Sigma I4753) preparation consisting of 95 units of invertase in 1 mL of 0.01 N acetate buffer (pH 4.5). Sucrose-glucose levels were converted to sucrose by multiplying by a factor of 1.9 (after first subtracting the free glucose values).

The starch-containing pellet was dried in a 55°C water bath, reconstituted in 3 mL H_2O and boiled for 2 h to gelatinize

the starch. Three mL of 0.1 N acetate buffer (pH 4.5) and 0.5 mL of amyloglucosidase (Sigma A9268) were added to the starch-containing fraction which was then incubated at 55°C for 24 h. After incubation, the tubes were spun at 9000*g* for 10 min and the supernatant retained for analysis. A glucose-oxidase kit (Sigma No. 510A) was used for glucose analysis in all cases. Starch-glucose values were multiplied by 0.9 to convert them to starch.

F-2,6-BP

Leaf discs were sampled by means of a light transmitting plexiglass punch into liquid N_2 over a diurnal period and stored at −80°C until extraction and assay. Two discs (7.88 cm^2) were homogenized in 1 mL of extraction buffer containing 75 mM Hepes (pH 8.1), 75 mM Tris (pH 8.1), 5 mM DTT, 5 mM EDTA, 10 mM NaF, and 10 mM NaH_2PO_4 in a liquid N_2-cooled mortar. The extract was transferred to a 1.5 mL Eppendorf tube, incubated immediately for 5 min at 80°C and centrifuged for 3 min in an Eppendorf centrifuge (model 5414). F-2,6-BP[1] was assayed according to Fahrendorf *et al.* (7). Sigma reagents of the highest quality were used throughout.

Enzyme Assays

All enzyme assays were made on crude leaf extracts sampled at 1, 6, and 16 h into a 16 h photoperiod and 30 min into the subsequent 8-h dark period. Sucrose-P-synthetase activity was determined on a crude leaf extract containing 2.77 cm^2 leaf·ml^{-1}. The extraction medium contained 50 mM Hepes (pH 7.5, NaOH), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, 1% PVP, and 1% BSA. The assay medium contained 50 mM Hepes (pH 7.5, NaOH), 7.5 mM UDP-Glucose, 7.5 mM fructose-6-P, 15 mM $MgCl_2$, and 50 μL of leaf extract in 0.5 mL according to the method of Rufty *et al.* (19).

A common leaf extract (3.32 cm^2 leaf mL^{-1}) was used for the assay of UDPG pyrophosphorylase, ADPG pyrophosphorylase, and cytosolic FBPase. The extraction buffer contained 50 mM Hepes (pH 7.5, NaOH), 5 mM $MgCl_2$, 1 mM EDTA, 1% PVP, and 1% BSA. DTT (5 mM) was added to the extraction medium for UDPG pyrophosphorylase and FBPase. GSH (2 mM) was found to optimize the activity of ADPG pyrophosphorylase, and therefore was added in the place of DTT which was inhibitory to the enzyme. The coupled enzyme assay procedures of Kerr *et al.* (12) were used for all three enzymes. A DW-2C SLM Aminco spectrophotometer was used in the dual wavelength mode (340 nm:380 nm) for the assays. Acid- and alkaline-invertases were assayed according to Ou-Lee and Setter (15). Alkaline-invertase activities were not detectable with the assay and therefore are not reported.

Total phosphorus determinations were made on perchloric:nitric (1:1 v/v) acid digests of replicate plant tissue samples using a sensitive method for P determination (13). Leaf Pi was estimated by homogenizing fresh leaf plugs in 2% acetic acid and extracting for 30 min before centrifuging at 4000*g* for 10 min (2).

[1] Abbreviations: F-2,6-BP, fructose-2,6-bisphosphate; ADPG, adenosine 5′-diphosphoglucose; FBPase, fructose-1,6-bisphosphatase; PSR, photosynthetic rate; SPS, sucrose-6-phosphate synthetase; UDPG, uridine 5′-diphosphoglucose.

RESULTS

Low-P treatment caused a significant decrease in the rate of shoot growth 7 d after treatment began (Fig. 1). Twenty-one d after transplant, low-P shoot dry weight was less than 17% of that of the high-P plants. In contrast, root dry weights were not affected by low-P until 17 d after transplant; at 21 d root dry weights were reduced by only 24% (data not shown). Plants grown with low-P partitioned dry matter between roots and shoots almost equally; plants with high-P partitioned nearly 5 times as much dry-matter to the shoot as to the root by d 21 (Fig. 1, inset).

Total leaf area was the parameter most highly affected by low-P treatment; it was decreased to less than 15% of the high-P value 19 d after transplant (Table I). This decrease in leaf area was accompanied by reductions of 67% in mean leaf area, 43% in leaf emergence, and 20% in specific leaf area.

Phosphorus concentrations in leaves, stems, and roots decreased dramatically with low-P treatment (Table II). Total leaf P and acetic-acid-soluble leaf P of P-stressed plants were 13 and 6% of the high-P values, respectively, 19 d after treatment began, while root total P was 12% of the high-P value. Total P concentrations were slightly higher in roots than shoots in both high-P and low-P plants; the total P content of roots of the low-P plants at 19 d was 157% of the shoot total P content compared to 54% for high-P plants (Table II).

Low-P treatment increased starch contents significantly in the uppermost fully expanded leaves; after 19 d of treatment, starch levels in the low-P leaves were at least double those of the high-P plants over the entire diurnal cycle (Fig. 2). Although the data show that the absolute levels of starch were higher in low-P leaves, the net rate of starch accumulation during the photoperiod was less in the low-P leaves; similarly, the net rate of starch degradation during darkness was also less in the low-P plants (as indicated by the average slopes of the data shown in Fig. 2). In contrast to starch, sucrose levels were less in low-P leaves (Fig. 3); the high levels of starch coupled with the low levels of sucrose resulted in much higher

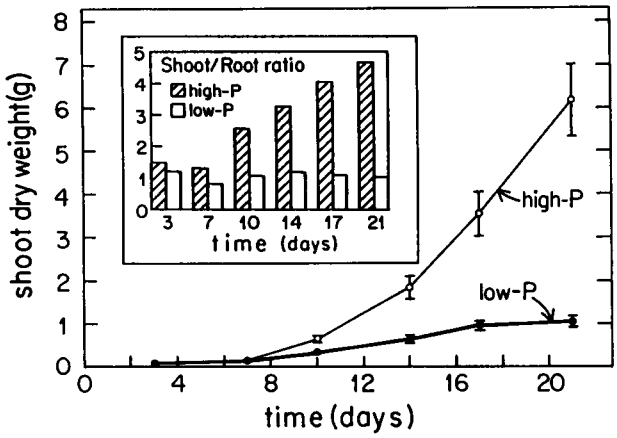

Figure 1. Shoot dry weights (g) and shoot/root dry weight ratios (inset) of soybeans grown with high-P or low-P (n=3, ±SD).

Table I. *Leaf Characteristics of Soybean Plants Grown with High-P or Low-P for 19 d after Transplant (n = 4, ±SD)*

Treatment	Specific Leaf Area	Total Leaf Area	No. Primary Trifoliates Initiated	Mean Trifoliate Area	Chl (a + b)
	$dm^2\ g^{-1}$ dry wt.	dm^2		dm^2	mg dm^{-2}
High-P	2.88 ± 0.17	12.1 ± 1.3	7	1.95 ± 0.72	3.02 ± .05
Low-P	2.01 ± 0.06	1.8 ± 0.2	4	0.65 ± 0.10	2.80 ± 0.21

Table II. *Phosphorus Concentrations in Leaves, Stems + Petioles, and Roots of Soybean Plants Grown With High-P and Low-P for 19 D After Transplant (n = 6, ±SD)*

Treatment	Total Leaf P	Leaf Inorganic P[a]	Organic P	Total Stem + Petiole P	Root P	Total Root P / Total Shoot P
			mg P g^{-1} dry wt.			
High-P	6.87 ± 1.59	4.43 ± 1.49	2.44	5.84 ± 0.84	10.65 ± 0.59	0.54
Low-P	0.87 ± 0.16	0.28 ± 0.06	0.59	1.14 ± 0.11	1.29 ± 0.05	1.57

[a] Two percent acetic acid soluble phosphorus.

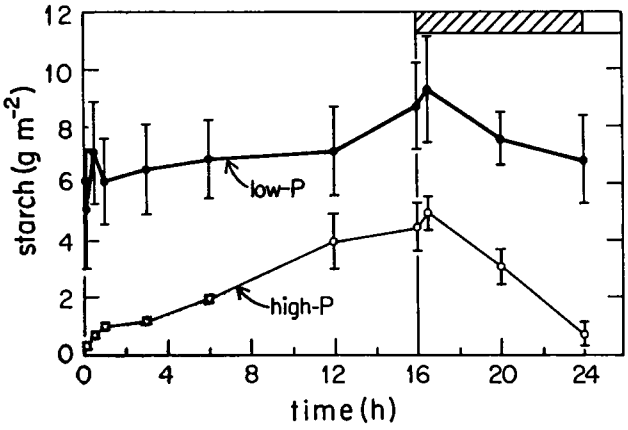

Figure 2. Starch contents of fully expanded leaves of soybeans grown with high-P or low-P for 19 d after transplant (*n*=3, ±SD).

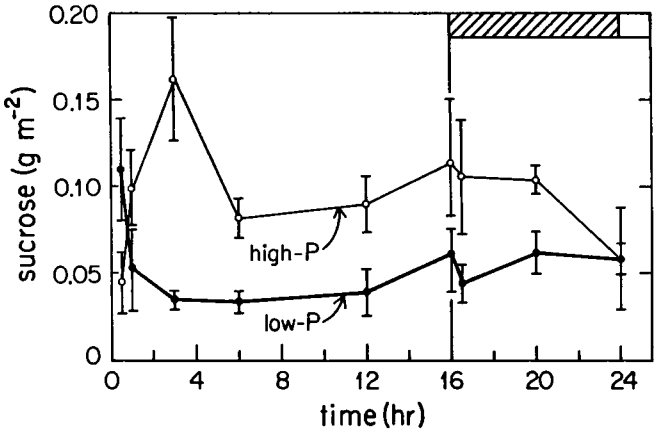

Figure 3. Sucrose contents of fully expanded leaves of soybeans grown with high-P or low-P for 19 d after transplant (*n*=3, ±SD).

starch/sucrose ratios in low-P compared to high-P plants throughout the diurnal cycle.

In addition to the data for the fully expanded leaves, starch and sucrose contents were also determined for rapidly expanding leaves (Table III). Rapidly expanding high-P leaves had very small amounts of starch. Low-P increased the starch contents of these younger leaves by 30-fold, suggesting that their growth was not limited by the supply of carbohydrate. Sucrose levels, however, were 4-fold lower in the low-P expanding leaves than in the high-P plants suggesting that sucrose imported into the young growing leaves was rapidly metabolized and incorporated into starch. In fibrous roots on the other hand, low-P treatment increased sucrose content 11-fold and starch 7-fold (Table III). The high concentration of starch in expanding leaves and roots and sucrose in roots of low P plants suggests that low-P treatment did not reduce growth by impairing carbon transport to the growing tissues.

The increase in starch in low-P leaves was accompanied by a 45% increase in the total activity of the starch synthesis pathway enzyme, ADPG pyrophosphorylase, in mature leaves

Table III. *Starch and Sucrose Contents of Expanding Leaves and Fibrous Roots of Soybean Grown with High-P and Low-P for 19 d after Transplant (n = 4, ±SD)*

Treatment	Expanding Leaf		Fibrous Root	
	Starch	Sucrose	Starch	Sucrose
	g CH_2O m^{-2} leaf		mg CH_2O g^{-1} fr wt	
High-P	0.4 ± 0.1	0.7 ± 0.1	23 ± 4	16 ± 10
Low-P	12.9 ± 0.7	0.20 ± 0.02	160 ± 19	177 ± 48

(Table IV). Low sucrose levels (in low-P leaves) on the other hand, were associated in varying ways with the total activities of sucrose-synthesizing enzymes. Low-P increased UDPG pyrophosphorylase total activity two-fold but had no effect on cytosolic FBPase activity. The total activities of UDPG pyrophosphorylase, ADPG pyrophosphorylase, and cytosolic FBPase did not vary diurnally (data not shown). SPS activity, however, did show diurnal variation. After 1 h of illumination, SPS decreased by 55% (Table IV). After 6 and 16 h of illumination, followed by 30 min of darkness, SPS levels were

Table IV. *Effect of P Treatment on the Total Activities of Certain Enzymes Involved in Sucrose and Starch Metabolism in Extracts from Mature Leaves of Glycine max after 1 h of Illumination (±SD)*

Enzyme	Low-P	High-P	% High-P
Cytosolic-FBPase	2.50 ± 0.22	2.43 ± 0.07	103
UDPG-pyrophosphorylase	157 ± 2	80 ± 3	196
Sucrose-P-synthetase	4.67 ± 2.3	10.5 ± 2.3	44
Acid-invertases	3.67 ± 0.33	2.17 ± 0.17	169
ADPG-pyrophosphorylase	4.33 ± 0.67	3.00 ± 0.50	144

Table V. *Activity of Sucrose-6-P Synthetase in Extracts from Mature Leaves of High-P or Low-P Glycine max 19 d after Transplant Sampled at 6 and 16 h into the Photoperiod and 30 min into the Subsequent Dark Period (n = 3, ±SD)*

Treatment	6 h light	16 h light	30 min dark
		$\mu mol\ m^{-2}\ s^{-1}$	
High-P	7.8 ± 1.7	5.9 ± 0.8	4.6 ± 0.5
Low-P	7.0 ± 2.2	4.7 ± 0.5	5.2 ± 1.1

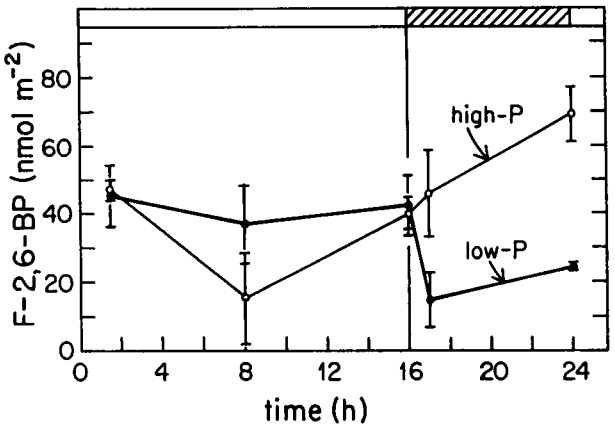

Figure 4. F-2,6-BP levels in fully expanded leaves of plants grown with high-P or low-P for 19 d after transplant (n=3, ±SD).

not affected by low P treatment (Table V). Low sucrose levels in low-P leaves may have been due in part to increased amounts of acid invertase; in mature leaves the total activity was increased by 69% (Table IV).

Foliar F-2,6-BP levels were similar in high-P and low-P plants over the 16-h light period (Fig. 4). In the subsequent 8-h dark period, levels of F-2,6-BP decreased in low-P plants and increased in high-P plants.

Photosynthesis irradiance response curves were determined for high-P and low-P plants at 19 d after transplant at ambient CO_2 (300 $\mu L \cdot L^{-1}$) and 21% O_2 (Fig. 5). Rates of photosynthesis at light intensities less than 250 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ were not affected by low-P treatment; at light saturation, photosynthesis was reduced by 55% by low-P treatment. The rate of photosynthesis at CO_2-saturation in low-P plants was reduced by 45% (Fig. 5, inset).

DISCUSSION

The most striking effect of low P treatment was the 85% reduction in total leaf area and the 78% reduction in shoot dry weight. When plants are deprived of P, decreases in

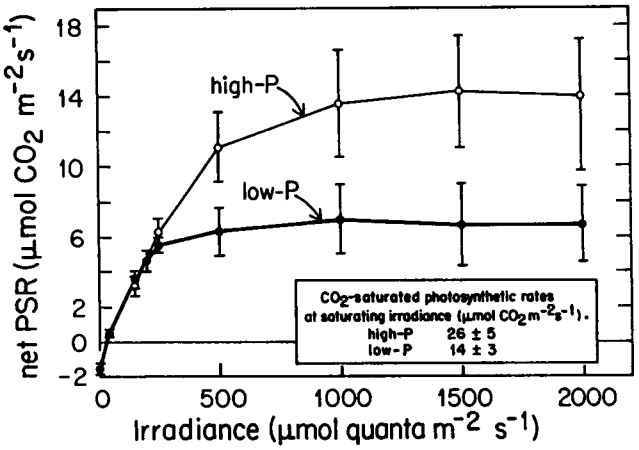

Figure 5. Irradiance response curves for photosynthesis of fully expanded leaves at ambient and saturating (inset) levels of CO_2 for soybeans grown with high-P or low-P for 18–20 d after transplant (n=3, ±SD).

photosynthetic (leaf) surface area are generally observed before the rate of photosynthesis per area is affected (1, 14). In the present work, the reduction in growth rate with low-P was not attributable to the decrease in photosynthetic rate: photosynthetic rates were decreased by only 43% (at 500 μmol quanta $\cdot m^{-2} \cdot s^{-1}$) and the accumulation of starch in young growing leaves (and of sucrose and starch in fibrous roots) indicated that there was no shortage of photosynthate in the low-P plants.

The reduction in the expansion of the leaf surface with low-P was mainly due to an effect on the expansion of individual leaves although there was some effect also on the rate of leaf emergence. Leaf expansion has been shown to be positively correlated with leaf epidermal cell area as the P status of cotton leaves is increased (18). Leaf epidermal cell expansion appears to be a critical process in the expansion of the leaf blade (28) and Pi concentrations in the upper epidermis are sharply reduced with decreased P supply to the leaf (26). We suggest that the marked reduction in leaf expansion which occurs with P deficiency may be due to an insufficiency of phosphate for the expansion of epidermal cells, phosphate being required for some important but unknown role in leaf epidermal expansion. P-nutrition might affect growth via cytokinin levels (20) and it has been suggested that Pi itself might regulate plant growth (27).

It is unclear why fibrous root growth was so little affected by low-P treatment. In low-P plants, photosynthate was partitioned equally between roots and shoots while in high-P plants the preponderance of fixed carbon went to the shoots. One possible explanation for this is that the proximity of the fibrous roots to the culture medium enabled them to draw preferentially on the supply of Pi available to the plant. Low P plants had a much greater proportion of their total P invested in their roots compared to shoots, *i.e.* root P/shoot P was 1.57 for low-P and 0.54 for high-P plants, and the roots of low-P plants had 82% higher P concentrations than the shoots. Roots undergo several adaptive changes in response to P stress. P uptake rates may increase following periods of P starvation: this may be due to some type of feedback effect of low plant P status on the activity of the Pi-transport protein,

to an increase in the quantity of the Pi-transport protein, or to morphological changes (4, 11). The increase in root/shoot ratio with low-P may also have been some kind of adaptive response in roots to low external levels of Pi.

Another explanation for the high rates of root growth in low-P plants is that low-P plants diverted more of their photosynthate toward roots because less was utilized in shoot growth. Certainly there were much higher levels of starch and sucrose in fibrous roots than leaves in low-P plants, indicating a considerable transport of photosynthate to roots had occurred. Other researchers have shown that C-export belowground as a fraction of total C-export from leaves is increased in plants supplied with suboptimal P (16).

Suboptimal P supply to plants *in vivo* generally results in enhanced levels of foliar starch (5, 9, 18). Here we observed increased levels of starch in young growing leaves, mature leaves, and fibrous roots of low-P plants. The simplest explanation of these results is that under P-limitation, more photosynthate was produced than could be utilized in growth, leading to higher levels of starch, and in fibrous roots, starch, and sucrose.

Sucrose levels in the fully expanded and expanding leaves of low-P plants were generally reduced below the levels observed in high-P plants. This was probably partly due to an increased acid-invertase activity in low-P leaves. The diminished pool sizes of sucrose were also associated with a decreased activity of the sucrose-synthesizing enzyme, SPS, but only at the first hour of the light period. At later times this effect of low-P treatment disappeared (an effect also observed by D. Israel with soybean [personal communication]).

Low-P treatment did not affect the extractable activity of cytosolic FBPase but did increase UDPG pyrophosphorylase. Why UDPG pyrophosphorylase activity should increase in low-P leaves while sucrose synthesis was apparently diminished is not clear. UDPG pyrophosphorylase forms UDPG which can be used in reactions involving the synthesis of cell wall polysaccharides (*e.g.* β-D-glucan synthase, UDPG-4-epimerase, and UDPG glucose dehydrogenase). If low-P treatment resulted in greater rates of synthesis of UDPG, this might in turn have led to an increased production of cell wall polysaccharides. There was some evidence for this since leaf dry weight increased more than could be accounted for by the increase in starch, *i.e.* specific leaf weight increased by 15 $g \cdot m^{-2}$ in low-P compared to high-P plants, with only 4 to 6 $g \cdot m^{-2}$ of the increase being attributable to increased starch. Thus, elevated UDPG pyrophosphorylase activity in low-P plants may be associated with increased structural carbohydrate production. The pyrophosphate released in the synthesis of UDPG could then be hydrolyzed to Pi, thereby increasing the availability of Pi for Pi-depleted plants.

Foliar F-2,6-BP levels have been shown to increase with the transition from light to dark (see Sicher *et al.* [21]). This also occurred in our experiments with soybean treated with high-P. However, in the low-P leaves, there was a decrease in F-2,6-BP levels with the light/dark transition. Despite this decrease in F-2,6-BP in low-P leaves, there was no accompanying change in starch/sucrose ratio over the same time interval (see Figs. 2 and 3). It is unclear why low-P treatment had no effect on F-2,6-BP in the light but decreased it in the dark (Fig. 4).

Low external Pi increases starch production in isolated chloroplasts (10, 23) and also enhances *in vitro* ADPG pyrophosphorylase activity (17). It has been suggested that low cytosolic Pi favors starch *versus* sucrose synthesis *in vivo* by diminishing the export of triose phosphate out of the chloroplast via the phosphate translocator (10). Foyer and Spencer (9) showed that starch/sucrose ratios were increased in leaves depleted of Pi nutritionally. There was also an increase in the starch/sucrose ratio with low-P treatment in the present work. However, this increase was associated with an increase in total extractable ADPG pyrophosphorylase activity, a transient decrease in SPS and an increase in acid-invertase activities. These observations suggest that the increase in starch/sucrose ratio may not necessarily have been mediated via the Pi-translocator but by changes in the activities of various enzymes involved in starch and sucrose metabolism. Fleck *et al.* (8) obtained data at variance with the role of the Pi-translocator as proposed by Heldt *et al.* (10); they found diminished starch/sucrose ratios in P-deficient wheat.

Research has shown that P deficiency affects photosynthesis via stomatal and nonstomatal components (2, 24). Nonstomatal limitation was clearly of importance in the present work because light-saturated photosynthetic rates were diminished to nearly the same extent by low-P at both ambient and saturating CO_2 levels.

ACKNOWLEDGMENT

We thank Clifford Carlson for assistance with the gas exchange measurements.

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