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Effects of phosphorus nutrition on photosynthesis in *Glycine max* (L.) Merr.

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Abstract. The effects of phosphorus nutrition on various aspects of photosynthetic metabolism have been examined for soybean plants (*Glycine max*) grown in growth chambers. Orthophosphate was supplied at two levels in 0.5-strength Hoagland's solution. At the end of the 19-d growth period, plants grown at 10 μM KH_2PO_4 (low-P plants) had undergone a 40% drop in net CO_2 exchange (averaged over a 16-h light period), as compared with control plants grown with 200 μM KH_2PO_4. Low-P resulted in reductions in the initial activities of five, and in the total activities of seven, Calvin-cycle enzymes. Notable exceptions were the initial and total activities of chloroplastic fructose-1,6-bisphosphatase (EC 3.1.3.11) which were increased by 85 and 53%, respectively, by low-P. Low-P decreased leaf 3-phosphoglycerate (PGA) levels most (by 80%), ribulose-1,5-bisphosphate (RuBP) less (by 47%) while triose-phosphate (TP) was not significantly changed. The results indicate that photosynthetic CO_2-fixation in low-P plants was limited more by RuBP regeneration than by ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39) activity. Ribulose-1,5-bisphosphate regeneration in low-P plants did not appear to be limited by ATP and-or NADPH supply because ATP/ADP and NADPH/$NADP^+$ ratios were increased by 60 and 37%, respectively, by low-P, and because TP/PGA ratios were higher in low-P plants. Low-P may diminish RuBP regeneration, and hence photosynthesis, by reducing Calvin-cycle enzyme activity, in particular, the initial activity of ribulose-5-phosphate kinase (EC 2.7.1.19) (44% reduction), and by enhancing the flux of carbon into starch biosynthesis.

Key words: Calvin cycle enzymes – *Glycine* (photosynthesis and P nutrition) – Phosphorus nutrition – Photosynthetic carbon metabolism

Introduction

Studies using isolated chloroplasts (and other in-vitro systems) have indicated that orthophosphate (Pi) may have an important role in photosynthetic metabolism (Furbank et al. 1987; Giersch and Robinson 1987). It may be involved in the activation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCase) (Heldt et al. 1978), modulation of ribulose-5-phosphate kinase, sedoheptulose-1,7-bisphosphate phosphatase, and fructose-1,6-bisphosphate phosphatase (Leegood et al. 1985), transport of triose-phosphate (TP) across the chloroplast membrane by the Pi-translocator (Flügge et al. 1980), and in the regulation of photophosphorylation (Flügge et al. 1980). When Pi is withheld from plants, there is a substantial non-stomatal inhibition of photosynthesis (Terry and Ulrich 1973; Brooks 1986; Fredeen et al. 1989; Rao and Terry 1989). It is not clear, however, how the effect of low phosphorus supply (low-P) on photosynthesis is mediated. Brooks (1986) demonstrated significant reductions in both RuBPCase activity and ribulose-1,5-bisphosphate (RuBP) regeneration in leaves of low-P-grown spinach and suggested that they co-limit photosynthesis. In sugar beet, low-P treatment appears to influence photosynthesis through RuBP regeneration rather than RuBPCase activity (Rao and Terry 1989; Rao et al. 1989a), indicating that low-P may affect photosynthetic rate differently with different species. In the present work we studied the effect of low-P treatment on photosynthesis in soybean by monitoring diurnal changes in photosynthetic CO_2 uptake, the activ-

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Abbreviations and symbols: AdN = adenine nucleotides (ATP + ADP + AMP); C_i = internal CO_2 concentration; NCE = net carbon exchange rate; NN = nicotinamide nucleotides (NADPH + $NADP^+$ + NADH + NAD^+); PFD = photosynthetically active photon flux density; PGA = 3-phosphoglyceric acid; RuBP = ribulose-1,5-bisphosphate; RuBPCase = ribulose-1,5-bisphosphate carboxylase/oxygenase; TP = triose phosphate

ities of nine Calvin-cycle enzymes, and diurnal changes in three leaf metabolites, RuBP, 3-phosphoglyceric acid (PGA) and TP. In addition, we evaluated the possibility that low-P influences RuBP regeneration through ATP and-or NADPH supply.

Material and methods

Plant material. Soybean plants (*Glycine max* (L.) Merr. cv. AM-SOY71; Agricultural Alumni Seed Improvement Association, Romney, Ind., USA) were grown hydroponically in controlled-environment growth chambers at 27/23° C (day/night) as described in Fredeen et al. (1989); a photosynthetically active photon flux density (PFD) of 525 ± 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was maintained over a 16-h light period. Seeds were germinated in vermiculite, and after 4 d the seedlings were transplanted into buckets (three seedlings per bucket) containing 20 L of 0.5-strength modified Hoagland's solution, the composition of which is given in Fredeen et al. (1989). Two different levels of P were supplied as KH_2PO_4: 200 μM (control), and 10 μM (low-P).

Gas exchange. Photosynthesis measurements were made on second trifoliates (numbered acropetally from the node of the first pair of single leaves) from low-P soybean plants, or third trifoliates from control plants, using steady-state gas-exchange equipment (Terry 1980) with $C_a = 0.31$ mmol $CO_2 \cdot mol^{-1}$ ($310\ \mu l \cdot L^{-1}$) and a PFD of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Leaf temperature was maintained at 25 ± 1° C throughout the light period for both control and low-P trifoliates. Photosynthesis measurements were replicated twice for control and low-P plants.

Sampling. Leaf discs ($3.88\ cm^2 \cdot disc^{-1}$) were sampled from both control (second and third trifoliate leaves) and low-P plants (first and second trifoliate leaves) 19 d after transplanting at appropriate intervals in the light and dark periods. Sampling of leaf discs was performed under similar illumination conditions for all leaves with a specially designed Plexiglas leaf puncher that did not interrupt ambient light (Rao et al. 1989b).

Chemicals. All chemicals for enzyme and metabolite assays were from Sigma Chemical Co., St. Louis, Mo., USA, except for the $NaH^{14}CO_3$ which was obtained from Amersham Corp., Arlington Heights, Ill., USA.

Enzyme assays. Activities of Calvin-cycle enzymes were determined by homogenizing two leaf discs for each replicate. In the control plant, one of the two leaf discs was taken from the second and one from the third trifoliate leaf. In the low-P plant, one leaf disc was removed from the first and one from the second trifoliate leaf. Extracts were made in a mortar and pestle cooled with liquid N_2 and 1 ml of the extract was centrifuged in a refrigerated Eppendorf (Brinkmann Instruments, Westbury, N.Y., USA) centrifuge (model 5414) for 3 min before assaying. Excepting RuBPCase (EC 4.1.1.39), initial activities were assayed immediately in the absence of dithiothreitol (DTT) in both extraction and assay media. The RuBPCase activity was assayed as described in Rao and Terry (1989). For the other enzyme assays, a common extraction medium was used, containing 100 mM Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, sodium salt] (pH 8.0), 10 mM $MgCl_2$, 100 mM Na-ascorbate, 0.4 mM EDTA (ethylenediaminetetraacetic acid), 1% w/v PVP (polyvinyl pyrrolidone), and 0.1% w/v BSA (bovine serum albumin). In addition, total-activity extracts contained 50 mM DTT (dithiothreitol). 3-Phosphoglycerate-kinase (EC 2.7.2.3), NADP-glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.13), ribulose-5-phosphate kinase (EC 2.7.1.19), chloroplastic fructose-1,6-bisphosphatase (EC 3.1.3.11), and sedoheptulose-1,7-bisphosphatase (EC 3.1.3.37) were assayed as described in Rao and Terry (1989). In the case of total sedoheptulose-1,7-bisphosphatase activity, extracts were desalted through a Sephadex (G-25M) column. Total activities of TP isomerase (EC 5.3.1.1), fructose-1,6-bisphosphate aldolase (EC 4.1.2.7), and transketolase (EC 2.2.1.1) were assayed as described in Rao and Terry (1989).

Foliar metabolites. Adenine nucleotide, RuPB, TP, and PGA levels were determined on the same extract. Leaf discs were sampled into liquid-N_2-filled crucibles under uninterrupted growth-chamber light conditions, ground into a fine powder, homogenized in 12% $HClO_4$ ($3.88\ cm^2 \cdot ml^{-1}$), and stored at −65° C. Extracts were neutralized with 5 N or 10 N KOH containing 0.35 M triethanolamine. Adenylate levels were assayed utilizing a firefly luciferin-luciferase bioluminescence method (Rao et al. 1989a, b) within 24 h of extract neutralization. Myokinase (EC 2.7.4.3) (Sigma M-3382) was desalted through a Sephadex (G-25M) column for the assay of AMP. Ribulose 1,5-bipshosphate, TP (dihydroxyacetone-phosphate + glyceraldehyde-3-phosphate) and PGA were determined as described before (Rao et al. 1989a). Neutralized $HClO_4$ extracts for assay of TP and PGA were clarified with activated charcoal ($10\ mg \cdot ml^{-1}$) before assay. Nicotinamide nucleotides (NN) were assayed according to Maciejewska and Kacperska (1987) with the following modification. Leaf discs were sampled as for the assays for other leaf metabolites. Either 1 N NaOH or 12% $HClO_4$ (3 ml) were added to the crucibles and the leaf powder was vigorously ground, providing four acid and four basic replicate samples at each time point for both control and low-P plants. Upon thawing, the contents were quantitatively transferred to tubes and stored at −65° C until analysis. Basic samples were neutralized with 6 N HCl and acidic samples were neutralized with 10 N KOH. Nicotinamide-nucleotide levels were assayed by enzyme cycling techniques with MTT [3-(4,5-dimethylthiazol-2-Y1-2,5-diphenyl-2H-tetrazolium bromide] as the terminal electron acceptor within 24 h of extract neutralization. Ethanol was spectroscopic grade. Glucose-6-phosphate dehydrogenase (type IX) (EC 1.1.1.49) derived from Bakers Yeast (Sigma, G4134) was chosen for low glutathione-reductase and "NADPH-oxidase" activity, so as not to interfere with determination of NADPH. The concentration of particular nicotinamide nucleotides in the extracts was calculated from appropriate standard curves obtained for standard NN solutions (see Rao et al. 1989b).

Results

Assimilation of CO_2. In control plants, net carbon-exchange rate (NCE) changed very little with time during the 16-h light period (Fig. 1A). The NCE was measured at a PFD of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (equivalent to the growth chamber PFD) which was below that needed for light saturation. In low-P plants, NCE was measured at the same PFD but in this case photosynthesis was not limited by light because photosynthesis of low-P leaves is saturated at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fredeen et al. 1989). In low-P plants, NCE appeared to increase over the first 2–3 h, then declined appreciably with time. This decline was almost certainly a consequence of decreased stomatal conductance: this is indicated by the fact that the internal CO_2 concentration (C_i) decreased with time in low-P leaves (Fig. 1B) so that the ratio of NCE/C_i in low-P plants exhibited no decrease with time (Fig. 1C). If one averages the NCE values over the light period for each treatment, the data show that low-P treatment decreased NCE by 40%. The average NCE/C_i ratio was decreased by 45% by low-P; thus, a major part of the reduction in photosynthesis with low-P was mediated through an effect on chloroplast function.

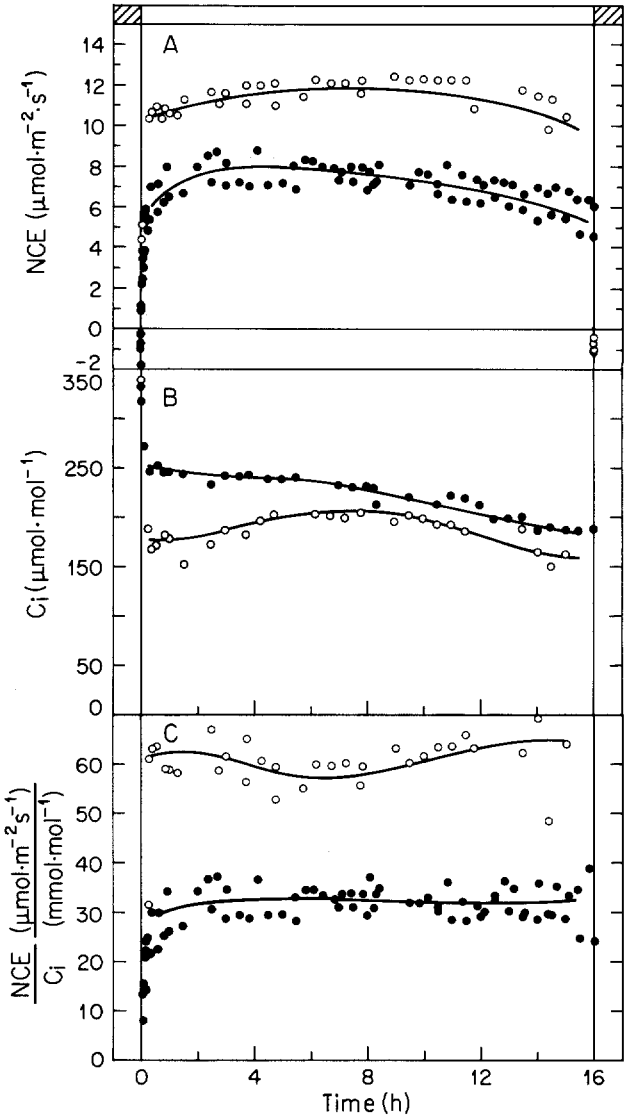

Fig. 1A–C. The effect of low-P (●) and control (○) on photosynthetic NCE (**A**), C_i (**B**), and NCE/C_i (**C**) over a 16-h light period in the leaves of soybean 19 d after transplanting. Each point represents one gas-exchange measurement

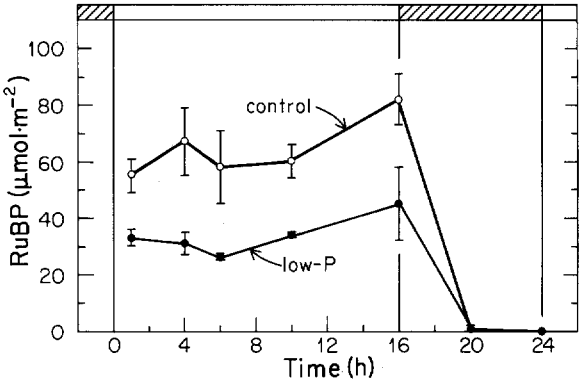

Fig. 2. The effect of low-P (●) and control (○) on diurnal foliar RuBP levels in the leaves of soybean 19 d after transplanting ($n=3$, ±SD)

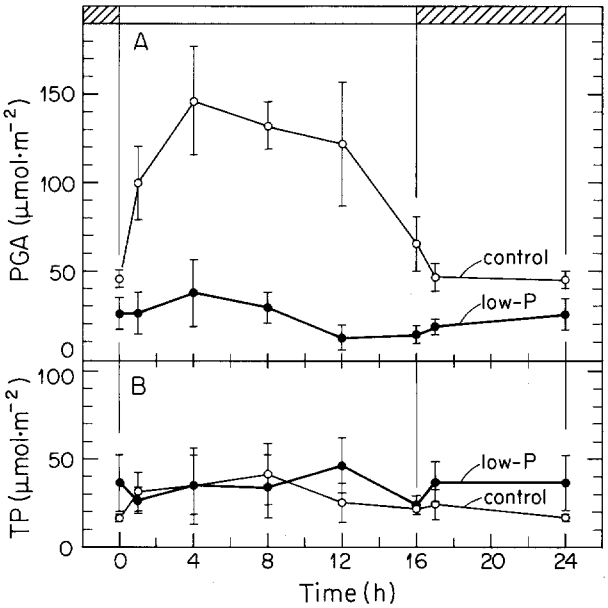

Fig. 3A, B. The effect of low-P (●) and control (○) on diurnal foliar PGA (**A**) and TP (**B**) levels in soybean 19 d after transplanting ($n=3$, ±SD)

Metabolite levels. Foliar RuBP levels changed little with time over the first 10 h of the light period; from 10 to 16 h there was a small increase in RuBP (not significant) in both control and low-P plants (Fig. 2). The average RuBP levels were 64 ± 11 and 34 ± 7 μmol·m^{-2} in control and low-P, respectively, i.e., low-P treatment decreased RuBP by 47%. Levels of RuBP were greatly diminished in the dark (to less than 1 μmol·m^{-2}) for both control and low-P plants.

Low-P treatment resulted in a very large reduction (approx. 80%) in leaf PGA levels in the light (Fig. 3A) but had no effect on leaf TP levels (Fig. 3B). Triosephosphate levels showed little diurnal variation with either treatment. Phosphoglycerate levels in low-P plants also exhibited little diurnal variation. In control plants, however, PGA levels increased from 45 to 145 μmol·m^{-2} during the first 4 h of light, then declined; the decline was most pronounced from 12 to 16 h (Fig. 3A). At the end of the light period (from 12 to 16 h), levels of PGA in control plants decreased nearly to dark levels; this decrease in PGA was associated with a small build-up in RuBP.

Activity of Calvin-cycle enzymes. Apart from chloroplastic fructose-1,6-bisphosphatase (and the total activity of sedoheptulose-1,7-bisphosphatase), low-P treatment decreased the initial (assumed to represent in-vivo activity) and total extractable activities of most Calvin-cycle enzymes (Table 1). The initial activities were usually more affected by low-P than were the total extractable activities. One exception to this was RuBPCase whose total activity was slightly more diminished by low-P than its initial activity; however, neither activity of this enzyme was much affected by low-P. Low-P treatment resulted in marked reductions in the initial activities of ribulose-5-phosphate kinase (44% decrease) and sedoheptulose-

Table 1. Effect of low-P and control on initial and total activities of selected Calvin-cycle enzymes in soybean leaves after 1.5 h of illumination at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PFD ($n=3$, $\pm$SD)

Enzyme Activity	Low-P ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Control ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Low-P as % of control
Ribulose-1,5-bisphosphate carboxylase/oxygenase			
Initial	90 ± 9	119 ± 14	76
Total	98 ±12	138 ± 6	71
3-Phosphoglyceric acid kinase			
Initial	47 ± 2	86 ± 3	55
Total	50 ± 9	64 ± 4	78
NADP-glyceraldehyde-3-phosphate dehydrogenase			
Initial	50 ±11	74 ± 2	68
Total	72 ±16	86 ± 13	84
Triose-phosphate isomerase			
Total	365 ±29	488 ±112	75
Fructose-1,6-bisphosphate aldolase			
Total	19.8 ± 2.2	24.4 ± 0.95	81
Fructose-1,6-bisphosphate phosphatase			
Initial	12.2 ± 0.4	6.6 ± 0.8	185
Total	15.3 ± 1.4	10.0 ± 0.9	153
Transketolase			
Total	5.06± 0.3	8.75± 0.5	58
Sedoheptulose-1,7-bisphosphate phosphatase			
Initial	1.88± 0.11	2.94± 0.17	64
Total	5.26± 0.5	4.37± 0.55	120
Ribulose-5-phosphate kinase			
Initial	39 ± 4	70 ± 1	56
Total	89 ± 14	95 ± 6	94

1,7-bisphosphatase (36%). The level of activation (initial activity/total activity) of Calvin-cycle enzymes was generally 70% or higher in both control and low-P plants; the activation states of sedoheptulose-1,7-bisphosphatase and ribulose-5-phosphate kinase in low-P plants were exceptions, exhibiting only 36% and 44% activation, respectively.

Chloroplastic fructose-1,6-bisphosphatase differed dramatically from the other enzymes in that its initial activity was increased 85% by low-P and its total activity by 53%. Total activity of sedoheptulose-1,7-bisphosphatase (assayed using desalted extracts) showed a slight increase of 20% with low-P. Low-P treatment has also been shown to increase the activities of these enzymes in sugar beet (Rao and Terry 1989).

Adenine and nicotinamide nucleotides. The levels of ATP, ADP and AMP were each reduced by low-P (Fig. 4). The ADP (Fig. 4B) and AMP (Fig. 4C) levels exhibited little change with time during the light period but ATP (Fig. 4A) levels tended to increase with time during the first 8 h of light. The total amount of adenylates (Fig. 4D) followed a similar course with time as a result of the influence of the changes in ATP which constituted a high proportion of the total adenylate pool. Low-P treatment, which decreased total adenylates by 44% on average and ATP levels by 54%, increased ATP/ADP ratios by 60%.

Low-P reduced the amounts of $NADP^+$ (Fig. 5A), NADPH (Fig. 5B) and NAD^+ (Fig. 5C), while NADH (Fig. 5D) was not affected. The total amount of all nicotinamide nucleotides did not change with time, the average values were about half that of the control value. The light-to-dark transition resulted in rapid (less than 1 h) decreases in NADPH and $NADP^+$ and a corresponding increase in NAD^+ levels to steady-state dark levels in both treatments. The reverse situation was observed in the dark-to-light transition when NADPH and $NADP^+$ levels increased concomitantly with a decrease in NAD^+ levels to steady-state light levels within 1 h of light. The NADH levels, which were not significantly affected by low-P treatment, exhibited no diurnal variations (Fig. 5D). The increase in ATP/ADP ratio with low-P treatment was accompanied by increases in reducing power: NADPH/$NADP^+$ and NADH/NAD^+ ratios averaged over the light period were 37% and 88% higher, respectively, in low-P compared to control plants.

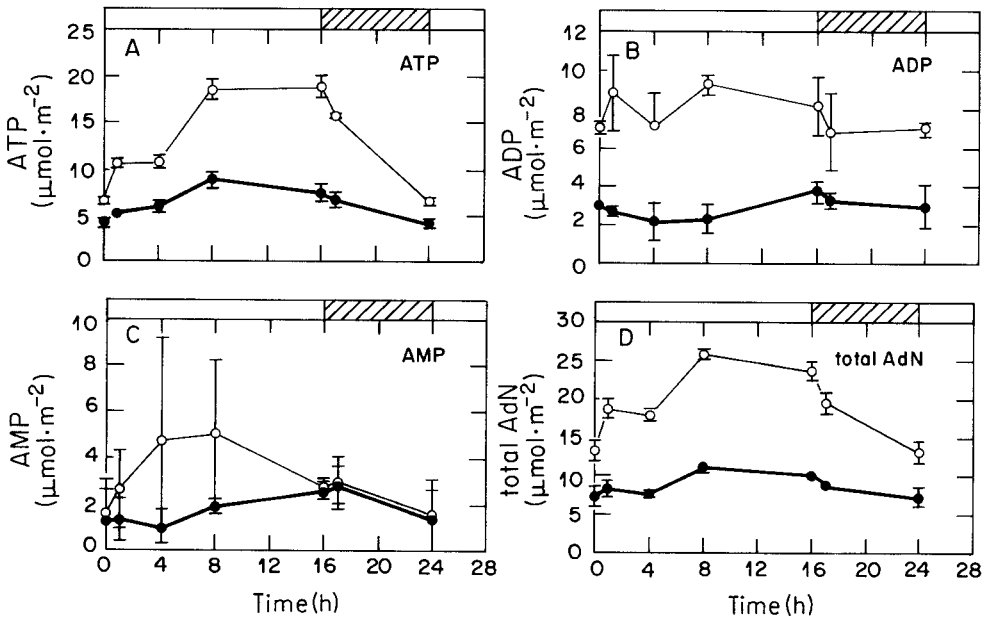

Fig. 4A–D. The effect of low-P (●) and control (○) on foliar adenine-nucleotide (*AdN*) levels over a 16-h light period in soybean 19 d after transplanting ($n=4$, $\pm$SD)

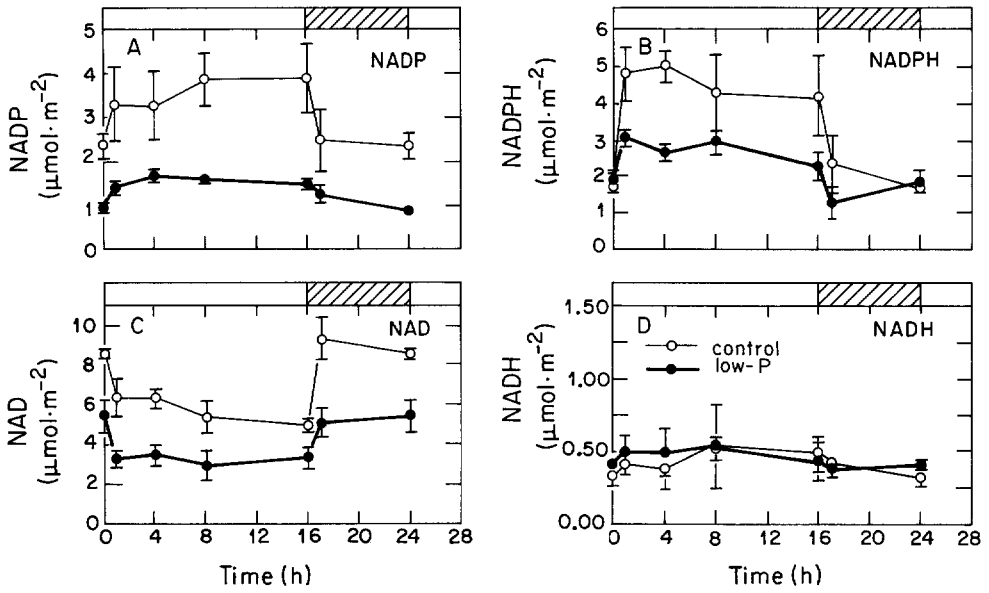

Fig. 5A–D. The effect of low-P (●) and control (○) on diurnal foliar nicotinamide-nucleotide levels in soybean 19 d after transplanting ($n=4$, ±SD)

The concomitant increase in ($NADPH+NADP^+$) and decrease in ($NADH+NAD^+$), which occurred in plants of both treatments during the dark-to-light transition, are thought to be a result of NAD-kinase (EC 2.7.1.23) activity. This is a largely chloroplastic enzyme which catalyses the light-dependent conversion of NAD^+ to $NADP^+$ (Muto and Miyachi 1985). Conversely, in the light-to-dark transition, a decrease in the combined ($NADPH+NADP^+$) pool paralleled the increase in the ($NADH+NAD^+$) pool in both control and low-P plants. The light-to-dark changes presumably resulted in part from the action of a chloroplastic "NADP phosphatase", an enzyme of which very little is yet known. The time-course of both the NAD^+ to $NADP^+$ and $NADP^+$ to NAD^+ conversions were comparable for both treatments, i.e., changes were complete within 1 h, somewhat faster than in the case of spinach (Bonzon et al. 1983).

Discussion

The results show that low-P treatment decreased photosynthetic rates by about 40% (at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PFD, 0.31 $mmol \cdot mol^{-1}$ ambient CO_2). Since the ratio of NCE/C_i was decreased 45% by low-P, it is clear that a substantial component of the effect of low-P on photosynthesis was nonstomatal in nature (see also Fredeen et al. 1989). Such non-stomatal limitation may be mediated through a reduction in the active sites of RuBPCase, or through a decrease in the rate of RuBP-regeneration. Ribulose-1,5-bisphosphate levels were reduced more by low-P (47% decreased) than the apparent activation of RuBPCase (24% decreased).

Calculation of the RuBPCase binding sites (assuming a K_{cat} of 3.25 mol $CO_2 \cdot$[mol enzyme sites$\cdot$s]$^{-1}$ for both control and low-P treatments; von Caemmerer and Edmondson 1986) shows that low-P treatment decreased the binding-site number by only 30%. It has been argued that RuBP concentrations should be close to twice the active site concentration to be non-limiting to RuBPCase activity (Woodrow and Berry 1988). Estimates of the [RuBP]/[binding site] ratio were 1.5 in control and 1.1 in low-P plants. We conclude therefore that RuBP regeneration is more limiting to photosynthetic CO_2-fixation in P-deficient plants than the decrease in RuBPCase activity (Brooks 1986; Brooks et al. 1988; Rao et al. 1989a). This conclusion is also in accord with the research of Sicher and Kremer (1988) who found that the initial and total activities of RuBPCase were increased by low-P in barley seedlings, while RuBP levels and CO_2 fixation in the same plants were decreased by 66% and 38%, respectively.

One possible cause for the reduction in RuBP regeneration with low-P treatment could be a decrease in ATP (and-or NADPH) caused by insufficient orthophosphate (Pi) for photophosphorylation (Giersch and Robinson 1987; Furbank et al. 1987). Our results show that low-P treatment did decrease foliar ATP and NADPH levels by 40%–50%. Nevertheless, it seems unlikely that RuBP regeneration was limited by ATP, or NADPH supply, because ATP/ADP and $NADPH/NADP^+$ ratios were increased by low-P treatment. The TP/PGA ratio was substantially increased by low-P, also indicating that there was sufficient ATP and NADPH for the reduction of PGA to TP. Research by Abadia et al. (1987) and Rao et al. (1989a) with sugar beet shows that photochemical capacity is not limiting to photosynthesis in low-P sugar-beet plants. Our results also indicate that the reduction in the initial activities of PGA kinase and NADP-glyceraldehyde-3-phosphate dehydrogenase which occurred with low-P could not have impaired the conversion of PGA to TP. It has been proposed that lower ATP levels found in low-P plants may reflect an overall reduction in inosine-5′-monophosphate pathway reactions as a consequence of a decrease in the precursor, ribose-5-phosphate (Rao et al. 1989a).

The reduction in RuBP regeneration with low-P treatment may have resulted from the decreased level of activation of key enzymes involved in the formation of RuBP from TP. One such enzyme is ribulose-5-phosphate kinase whose initial activity decreased by 44% in response to low-P. Another enzyme which may be important in this respect is sedoheptulose-1,7-bisphosphatase whose initial activity was decreased 36%. Both these enzymes exhibited low levels of activation (approx. 40%) compared to the other enzymes assayed (>70%), indicating that their in-vivo activities may have been rate-limiting.

The reduction in RuBP regeneration with low-P may also have been the result of an increased utilization of chloroplast sugar-phosphates in starch biosynthesis (Fredeen et al. 1989; Rao et al. 1990). That sugar-phosphates from the Calvin-cycle may have been "drained-off" into starch formation is indicated by the fact that total activities of enzymes in the starch-forming pathway were increased. Chloroplastic fructose 1,6-bisphosphatase was increased by 85% by low-P and adenosine 5-diphosphoglucose pyrophosphorylase by 50% (Fredeen et al. 1989). Increased levels of starch were found in low-P soybeans (Foyer and Spencer 1986; Fredeen et al. 1989), supporting the view that the increased starch was the result of an enhanced capacity for starch synthesis. In addition, we believe that the 42% reduction in total transketolase activity in low-P, would accentuate the effects of elevated chloroplastic fructose-1,6-bisphosphatase and ADP-glucose pyrophosphorylase activities, resulting in further enhancement of the conversion of fructose-6-phosphate into starch. Increased production of starch would not only lower the levels of Calvin-cycle intermediate but could also presumably conserve stromal Pi under Pi-limiting conditions.

Phosphoglyceric-acid pools were depleted by about 80% in the low-P treatment, the largest effect among any of the phosphorylated intermediates we measured. Large reductions in PGA pool size in low-P plants have been observed for spinach (Dietz and Foyer 1986; Brooks et al. 1988), barley (Dietz and Foyer 1986; Sicher and Kremer 1988) and sugar beet (Rao et al. 1989a). The low PGA levels in low-P leaves, which were correlated with elevated ATP/ADP, $NADPH/NADP^+$ and TP/PGA ratios, indicate that the PGA reduction efficiency in low-P leaves was higher than the control leaves. The high PGA levels found in the light in control plants indicate that the rate of PGA formation exceeded the rate of PGA reduction and that therefore the rates of ATP and NADPH formation are limiting PGA reduction.

In conclusion, our results support the view that low-P diminishes photosynthesis in soybean by reducing RuBP regeneration, and not through effects on RuBPCase activity or activation state. We show that this effect of low-P on RuBP regeneration is not mediated through decreased phosphorylation or reducing power, but is more likely the result of a decrease in the level of activation of key enzymes involved in RuBP regeneration, e.g., ribulose-5-phosphate kinase and sedoheptulose-1,7-bisphosphatase.

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