Whole Leaf Carbon Exchange Characteristics of Phosphate Deficient Soybeans (*Glycine max* L.)

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ABSTRACT

Low phosphate nutrition results in increased chlorophyll fluorescence, reduced photosynthetic rate, accumulation of starch and sucrose in leaves, and low crop yields. This study investigated physiological responses of soybean (*Glycine max* [L.] Merr.) leaves to low inorganic phosphate (Pi) conditions. Responses of photosynthesis to light and CO₂ were examined for leaves of soybean grown at high (0.50 millimolar) or low (0.05 millimolar) Pi. Leaves of low Pi plants exhibited paraheliotropic orientation on bright sunny days rather than the normal diheliotropic orientation exhibited by leaves of high Pi soybeans. Leaves of plants grown at high Pi had significantly higher light saturation points (1000 versus 630 micromole photons [400–700 nanometers] per square meter per second) and higher apparent quantum efficiency (0.062 versus 0.044 mole CO₂ per mole photons) at ambient (34 pascals) CO₂ than did low Pi leaves, yet stomatal conductances were similar. High Pi leaves also had significantly higher carboxylation efficiency (2.90 versus 0.49 micromole CO₂ per square meter per second per pascal), a lower CO₂ compensation point (6.9 versus 11.9 pascals), and a higher photosynthetic rate at 34 pascals CO₂ (19.5 versus 6.7 micromoles CO₂ per square meter per second) than did low Pi leaves. Soluble protein (0.94 versus 0.73 milligram per square centimeter), ribulose-1,5-bisphosphate carboxylase/oxygenase content (0.33 versus 0.25 milligram per square centimeter), and ribulose-1,5-bisphosphate carboxylase/oxygenase specific activity (25.0 versus 16.7 micromoles per square meter per second) were significantly greater in leaves of plants in the high Pi treatment. The data indicate that Pi stress alters the plant's CO₂ reduction characteristics, which may in turn affect the plant's capacity to accommodate normal radiation loads.

Walker and Sivak (29) reviewed a set of plant attributes that respond to low Pi nutrition, which they term "the syndrome of Pi deficiency." The responses to low Pi include increased oscillatory behavior in *A* at lower light levels,

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3 Abbreviations: *A*, net carbon assimilation rate; RuBP, ribulose-1,5-bisphosphate; C₅, internal CO₂ concentration; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39).

increased light scattering within leaves, highly energized chloroplasts, and light saturation of *A* at lower light intensities. Response to low Pi nutrition may be somewhat variable among species, as levels of Pi that reduce *A* in sucrose-accumulating species may show little influence on starch-accumulating species (11, 29). Formation of starch liberates Pi from reduced carbon, thereby making Pi available for other reactions. As Pi is itself a key metabolite, a component of every compound in the Calvin cycle, every metabolite in the sucrose synthesis pathway, and a key part of the adenylates and nucleotides, the maintenance of some minimal level of Pi in the cytosol is critical for maintenance of normal plant metabolism.

While much information has been gained by examining isolated chloroplasts bathed in varying amounts of Pi (15, 27, 29), less is known regarding the response of intact plants to Pi stress. Under Pi deficiency, *A* may (6, 11, 12, 22) or may not be reduced (11), fluorescence may (6) or may not increase (1), and sucrose may (13) or may not accumulate (12). RuBP pool size decreased (6) and starch accumulated in a starch accumulator such as soybean (12, 13, 19). In addition, the quantum efficiency of spinach leaves decreased (6), but that of soybean leaves was not affected by low Pi nutrition (12). Due to the variability in these responses, further study is needed to better characterize intact plant responses to altered Pi nutrition.

One way to examine the response of intact leaves to low Pi is to examine *A* at varying PPFD and *C₅*. Pi stress is associated with increased oscillation in CO₂ fixation capacity of chloroplasts and increased fluorescence of Chl at low light (27), suggesting that low Pi limits *A* (29) and that quantum efficiency might be affected. The response of *A* at low light can help explain whole leaf response to low Pi nutrition. It has been reported that low Pi decreases the PPFD saturation point and *A* at PPFD saturation for soybean leaves (12). The response of *A* to *C₅* allows the partitioning of various limitations to *A* and permits the calculation of such parameters as carboxylation efficiency and the CO₂ compensation point (10, 16). This research was initiated to further examine the effects of Pi nutrition on carbon fixation and light response of photosynthesis of soybean leaves after preliminary observations that leaves of Pi-stressed soybean exhibited paraheliotropicism rather than the normal diheliotropic response of high Pi legumes including soybean.
MATERIALS AND METHODS

Plant Culture

Soybean (Glycine max [L.] Merr. cv Williams 82) plants were grown for 5 to 6 weeks in the greenhouse in sand culture in 20 L pots using nutrient solution prepared as described elsewhere (19), except to contain either 0.5 mM Pi or 0.05 mM Pi. Photographs to document leaf heliometric response were taken with two 35 mm cameras which were placed at equal distances from the high and low Pi plants. Two days before carbon exchange measurements, plants were moved to a growth chamber to acclimate to the following conditions: 28/20°C day/night, 400 μmol PPFD·m⁻²·s⁻¹ for 14 h, and 60 to 70% RH. For photosynthetic measurements, leaves were selected that had begun to expand simultaneously on the low and high Pi treatments because the low Pi treatment had a slower rate of leaf expansion than the high Pi treatment. Measurements commenced when the low Pi leaf reached full expansion.

Gas Exchange Measurements

Net photosynthesis was measured in an open gas exchange system on terminal trifoliolate leaflets or entire leaves enclosed in a pellet-cooled cuvette. Leaf and air temperatures were measured with 0.013 mm and 0.50 mm copper-constantan thermocouples, respectively. Air of varying CO₂ concentration was prepared by injection of CO₂/N₂ (8% v/v) into air scrubbed free of CO₂ by passage over soda lime. After humidification by bubbling through water, the air stream was passed through a temperature-controlled, water-cooled jacket to set an exit dew point of 10 to 13°C. Absolute CO₂ concentration of air passing to the cuvette and the difference in CO₂ concentration between entry and exit air streams was monitored continuously with Beckman 215B (Beckman Instruments, Fullerton, CA) and ADC model 225 Mark III (Analytical Development Corporation, Ltd, Hoddesdon, England; distributed by P. K. Morgan Inst. Co., Andover, MA) infrared gas analyzers, respectively, calibrated with primary standard gases (Matheson Gas Products, Joliet, IL). Dew point of entry and exit air streams was measured with two EG&G chilled-mirror dew point hygrometers (models 880 and 911, EG&G Instruments, Waltham, MA). Outputs of all instruments were sampled and logged by a microcomputer-based data acquisition system. For PPFD response measurements, a multiwatt lamp (GE E-040M, General Electric, Hendersonville, NC) was elevated to different heights above the cuvette or neutral density screening was placed between the lamp and cuvette.

A, Ci, and stomatal conductance were calculated using the equations of Farquhar and Sharkey (10). Since the slopes of the A versus C curves were linear, stomatal limitation was estimated as (Cᵢ − Cᵢ)/[Cᵢ − I], where Cᵢ = the ambient [CO₂], Cᵢ = the internal [CO₂], and I = the CO₂ compensation point (16). The CO₂ compensation point was taken as the x-intercept of the A versus Cᵢ relationship. Carboxylation efficiency was estimated as the slope of the linear part of the A versus Cᵢ relationship after a regression line was fitted to the data. Light saturation data were gathered after plants had acclimated to the growth chamber for 6 d. The point of light saturation was considered to be the PPFD needed for 95% of maximum A. Apparent quantum efficiency (i.e., on an incident PPFD basis) was estimated as the initial slope of the A versus PPFD relationship near the light compensation point.

Autoradiography

Autoradiograms of leaves of the 0.50 and 0.05 mM Pi treatments were developed to investigate for evidence of non-uniform stomatal opening. Leaf material of each treatment was allowed to equilibrate at 37 Pa CO₂ in a cuvette illuminated with a T-3 series tungsten halogen lamp (Regent Lighting Corp., Burlington, NC) at a PPFD of 850 μmol·m⁻²·s⁻¹ at the leaf surface. The radiation was filtered through 5 cm of water to reduce the heat load on the leaf during equilibration and measurement. After 20 min equilibration, ¹⁴CO₂ was released into the sealed cuvette by acidification of [¹⁴C]NaHCO₃ with 1 N HCl, and leaves were exposed for 1 min before purging cuvette air through soda lime. Air temperature during labelling was 27.6°C. Leaves of the 0.50 and 0.05 mM Pi treatments were exposed to 10 and 15 μCi ¹⁴CO₂, respectively. After the cuvette was purged, the leaf was rapidly excised and placed between two 0.64 cm aluminum plates that had been prechilled in liquid N₂. X-ray film was developed after being exposed to the samples for 6 d at −80°C.

Protein and Rubisco Assay

Leaf discs (1.62 cm²) from both treatments were harvested at midday with a No. 10 cork borer, immediately frozen in liquid N₂, and transported to the laboratory. The Rubisco assay was a modification of the method of Joseph et al. (17) and D. McDermitt (Li-Cor Inc, Lincoln, NE, personal communication). Ten discs were ground to a powder in liquid N₂ and then further ground in 10 mL of cold grinding buffer (100 mM Bicine-NaOH, 20 mM KCl, 5 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, 5 μM leupeptin, 1 mM ε-aminocaproic acid, and 1 mM benzamidine [pH 8.0]) on ice for 3 min. An aliquot was centrifuged for 1 min, then 50 μL were activated for 6 min at room temperature in assay buffer (100 mM Bicine, 22 mM KCl, 24 mM MgCl₂, 1.67 mM DTT and 2.5 mM [¹⁴C]NaHCO₃ (specific activity = 0.68 Ci·mol⁻¹; Amersham, pH 8.2) (17) (D McDermitt, personal communication). The assay was initiated by injecting 10 μL of 10 mM RuBP into the assay mixture and stopped after 30 s by injecting 50 μL of the complete assay mixture into 3 N HCl in methanol. Samples were evaporated to dryness and redissolved in 100 μL of H₂O. Four mL of 3a70B scintillation cocktail (Research Products International, Mount Prospect, IL) were added, and the samples were counted. The measured activities were multiplied by 1.05 to account for discrimination of Rubisco against ¹⁴CO₂ (24). The in vitro Rubisco activities were lower than the measured rates of intact leaf photosynthesis at high [CO₂]; therefore, comparisons of Rubisco activity between treatments were made on the assumption that the reduction in activity was the same for both treatments. The same aliquot used for the assay of Rubisco activity was used for the determination of soluble protein (5) and Rubisco amount, which was measured by rocket immunoelectrophoresis (17). Chl content was determined on a set of duplicate discs using the
method of Wintermanns and DeMots (30). Except where indicated, all chemicals were obtained from Sigma Chemical Company, St. Louis, MO. Regression lines were generated by computer fit to the raw data. Data were analyzed by analysis of variance.

RESULTS

Observation of Paraheliotropism

Leaves of plants grown on 0.50 mM Pi or 0.05 mM Pi were photographed to monitor their orientation throughout the day (Fig. 1). Leaves of many plants exhibit diapheliotropism (9) (solar tracking), but on bright sunny days, leaves of low-Pi soybeans assumed paraheliotropic (leaflet laminae parallel to incident radiation) positions. The leaves of the plants treated with 0.50 mM Pi were diapheliotropic throughout the day (Fig. 1a–c), following the arc of the sun through the sky. Leaves of the 0.05 mM Pi-treated plants were diapheliotropic both early and late in the day (Fig. 1, d and f), but exhibited paraheliotropic orientation at mid-day when subjected to PPFD levels exceeding about 1100 μmol·m⁻²·s⁻¹ (Fig. 1e). The paraheliotropic response of low Pi leaves was observed only on sunny days.

Response of A to Light

Because leaves of low-Pi soybeans showed light avoidance, a series of light response curves (A versus PPFD) of leaves constrained to be perpendicular to incident light were constructed for both treatments (Fig. 2). Light saturation was achieved at 630 μmol·m⁻²·s⁻¹ versus 1000 μmol·m⁻²·s⁻¹ PPFD for plants grown on 0.05 and 0.50 mM Pi, respectively. Photosynthesis at 95% saturating PPFD and 34 Pa CO₂ was 10.5 μmol·m⁻²·s⁻¹ for the low Pi treatment and 21.4 μmol·m⁻²·s⁻¹ for the high Pi treatment. In this experiment, the initial slope of the light response curves represents the apparent quantum efficiency because the assimilation rate data were plotted against PPFD incident upon the surface of the leaf rather than that actually absorbed. The apparent quantum efficiencies were 0.062 and 0.044 mol CO₂ fixed per mol photons for the high and low Pi treatments, respectively.

Response of A to [Cᵢ]

To further evaluate carbon reduction characteristics under low Pi, a series of A versus Cᵢ curves were generated on another set of plants (Fig. 3). We were only able to flow air containing up to 60 Pa CO₂ to the cuvette for measurement of A versus Cᵢ. Under our measurement conditions, we obtained a curvilinear A versus Cᵢ relationship for the 0.50 mM Pi plants and a linear A versus Cᵢ relationship for the 0.05 mM Pi plants. Parameters derived from A versus Cᵢ relationships for 0.50 mM Pi- and 0.05 mM Pi-treated plants (Table I) indicated that A at an external CO₂ partial pressure of 34 Pa was reduced from 19.5 to 6.7 μmol·m⁻²·s⁻¹ by low Pi culture. A decrease in A was reported previously for Pi-stressed soybean (12), spinach (6, 18), sugar beet (22), and cotton (2). The decrease in A was associated with significantly reduced carboxylation efficiency when soybean was grown at 0.05 rather than 0.50 mM Pi (Table I). An internal CO₂ partial pressure of only 15.9 Pa was observed for the 0.50 mM Pi leaves, whereas that of the 0.05 mM Pi leaves was 26.2 Pa.

Although stomatal conductance did not differ between treatments, stomatal limitation of photosynthesis was significantly greater for the 0.50 mM Pi treatment than for the 0.05 mM Pi treatment (Table I). Greater stomatal limitation to A would occur under conditions of high mesophyll photosynthetic potential, but relatively low stomatal conductance. The lower value for stomatal limitation to A of the low Pi treatment implies that factors other than stomatal conductance are more limiting to photosynthesis than are the stomata.

Protein, Rubisco and Chi

Leaves of the low Pi treatment had less protein (0.73 mg·cm⁻²) than high-Pi leaves (0.94 mg·cm⁻²) (Table II). The fraction of soluble protein that consisted of Rubisco was similar between treatments (34.7% of the soluble protein), therefore, the significant decrease in protein also reflected a reduction in the amount of Rubisco per unit leaf area (Table II). As plants of both treatments were dependent on fixed nitrogen for N, specific activity of Rubisco under the assay conditions should have been comparable unless the enzyme itself was affected by treatment. The measured specific activity of Rubisco decreased 33% when plants were grown with 0.05 mM Pi. Low Pi spinach also had lower Rubisco activity on an area basis, and the enzyme had a lower level of activation (6). Chi content of the leaves decreased 11% when soybeans were grown on 0.05 mM Pi rather than 0.50 mM Pi. Specific leaf weight of plants of the 0.50 mM Pi treatment was less than that of the 0.05 mM Pi treatment plants (2.97 versus 4.06 mg·cm⁻²), as has been been reported previously for low Pi soybeans (12). Part of this increase may be due to accumulation of starch (12, 13, 19).

Autoradiography

The validity of information derived from A versus Cᵢ relationships has recently been challenged by evidence of nonuniform stomatal closure (28). The A versus Cᵢ analysis assumes that the leaf is behaving in a generally uniform manner. Leaf tissue of the 0.50 and 0.05 mM Pi treatments were allowed to fix ¹⁴CO₂ for 1 min, then quickly frozen to prevent metabolite movement, and exposed to x-ray film. Nonuniform stomatal opening would appear as pale sectors bounded by vascular tissue. The autoradiograms showed no evidence of nonuniform stomatal opening in leaf tissue of soybean that had been grown on 0.50 or 0.05 mM Pi (Fig. 4). Therefore, the interpretation of the A versus Cᵢ relationship data was not affected by nonuniform stomatal opening.

DISCUSSION

The low Pi-treated leaves appeared to be actively avoiding direct radiation during the period of maximum solar intensity (Fig. 1). It is suggested from this pattern of leaf movement that under low Pi nutrition soybean leaves are unable to accommodate high radiation (9). The paraheliotropic behavior of low Pi leaves may mean that plants are capable of
Figure 1. Heliotropic response of soybeans grown on 0.50 mM Pi (left) or 0.05 mM Pi (right). The photographs were taken at 8 a.m. (a, d), 1 p.m. (b, e) and at 5 p.m. (c, f) on a sunny day with cameras mounted south of the plants (east to the right of the photograph, west to the left). Note the leaflet movements of the low Pi treatment resulting in lower interception of direct sunlight, particularly at mid-day. The light avoidance response of the low Pi treatment was observed only on bright sunny days.
grown plants were measured. Each datum represents a single determination.

Figure 2. Light response curve (A versus PPFD) for the 0.50 (open squares) and 0.05 mM Pi (closed circles) treatments. Greenhouse grown plants were allowed to adapt to the growth chamber for 6 d before measurement. Leaves were oriented perpendicular to the light source during measurement. Four leaves of each treatment were measured. Each datum represents a single determination.

Figure 3. Net CO₂ assimilation rate versus leaf internal CO₂ concentration response curves for the 0.50 (open squares) and 0.05 mM Pi (closed circles) treatments. The arrows indicate the point on the curves which correspond to the mean photosynthetic rate of 34 Pa CO₂. Greenhouse grown plants were allowed to adapt to the growth chamber for at least 48 h before measurement. Three separate experiments representing a total of eight leaves from each treatment were measured. Leaves were illuminated with saturating PPFD (>1100 μmol·m⁻²·s⁻¹). Each datum represents a single determination.

responding at the organ level to the stress associated with low Pi and high light. A similar paraheliotropic response has been observed for waterstressed soybeans subjected to high light (4).

When measured at 34 Pa CO₂, growth of soybean on 0.05 mM Pi reduced A 51% and the point of light saturation 37% (Fig. 2) relative to growth on 0.50 mM Pi. Lower A at light saturation and a lower point of light saturation have been

Table I. Summary of Phosphate Effects on Assimilation Rate versus Internal CO₂ Content of Soybean Leaves

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment (mM Pi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>Photosynthetic rate (at 34 Pa CO₂; μmol·m⁻²·s⁻¹)</td>
<td>19.5*</td>
</tr>
<tr>
<td>Stomatal conductance (at 34 Pa CO₂; mmol·m⁻²·s⁻¹)</td>
<td>174</td>
</tr>
<tr>
<td>Stomatal limitation (at 34 Pa CO₂; unlitless)</td>
<td>0.53</td>
</tr>
<tr>
<td>Carboxylation efficiency (μmol·m⁻²·s⁻¹·Pa⁻¹)</td>
<td>2.90</td>
</tr>
<tr>
<td>Internal [CO₂] (at 34 Pa external CO₂; Pa)</td>
<td>15.9</td>
</tr>
<tr>
<td>CO₂ compensation point (Pa)</td>
<td>6.94</td>
</tr>
</tbody>
</table>

* Data are the means of three separate experiments with a total of eight replications. ** Not significant. ** Denotes significance at the 0.01 confidence level.

Table II. Phosphate Effects on Soybean Leaf Soluble Protein, Percent Rubisco, Rubisco Specific Activity, Chl Content, and Specific Leaf Weight

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment (mM Pi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>Soluble protein/area (mg·cm⁻²)</td>
<td>0.94*</td>
</tr>
<tr>
<td>Rubisco/area (mg·cm⁻²)</td>
<td>0.33</td>
</tr>
<tr>
<td>Rubisco/soluble protein (%)</td>
<td>34.8</td>
</tr>
<tr>
<td>Specific activity, rubisco</td>
<td>25.0</td>
</tr>
<tr>
<td>(μmol·m⁻²·s⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Chl/area (μg·cm⁻²)</td>
<td>38.3</td>
</tr>
<tr>
<td>Specific leaf weight (mg·cm⁻²)</td>
<td>2.97</td>
</tr>
</tbody>
</table>

* Data are the means of three separate experiments with a total of eight replications. ** Not significant. ** Denotes significance at the 0.01 confidence level.

Figure 4. Autoradiograms of leaf tissue from the 0.50 mM Pi treatment (left) and the 0.05 mM Pi treatment (right). Leaf tissue was exposed to [¹⁴C]CO₂ for 1 m. x-ray film was exposed to the quickly frozen tissue for 6 d, and the negatives were printed simultaneously. The lighter intensity of the autoradiogram of the 0.05 mM Pi treatment leaf is due to lower A.
reported for low Pi versus high Pi soybeans (12) and a starchless mutant of Arabidopsis (26). Pi feeding to the Arabidopsis mutant increased both A and the point of light saturation. Although Fredeen et al. (12) detected no effect of low Pi on quantum efficiency in soybean, Brooks (6) found reduced quantum efficiency for low Pi spinach leaves, but detected no effect on spectral properties of leaves or Chl content. Chl content was reduced 11% in our study (Table II), but this may not entirely account for the 40% decrease in apparent quantum efficiency or the 51% reduction in light-saturated photosynthesis at 34 Pa CO₂. The suggestion that Pi limitation has a greater effect on the carbon reduction reactions than on the light reactions (1, 12, 26, 27) seems supported by the relatively greater reduction in A than in the light saturation point.

The A versus Cᵢ relationships for the 0.50 and 0.05 mM Pi treatments were strikingly different (Fig. 3). Under high Pi, the A versus Cᵢ curve was curvilinear, reflecting a shift from a CO₂-limited toward a CO₂-saturated condition at about 17 Pa CO₂. Leaves of plants grown on 0.05 mM Pi showed no change in the slope of the A versus Cᵢ relationship as Cᵢ increased well above 35 Pa. The nonvacular Pi concentration of similarly grown low Pi soybeans was estimated to be 0.23 mM versus 8.32 mM for high Pi plants (19). Due to the culture conditions under low Pi, low Pi leaves would appear to be Pi limited rather than CO₂ limited. Low Pi nutrition may lead to the development of chronic Pi limitation of A, even under low Cᵢ. The low carboxylation efficiency of the low Pi leaves (Table I) indicated that carbon fixation at low Cᵢ was not functioning as efficiently as in high Pi leaves.

The greater carboxylation efficiency of leaves of the 0.50 mM Pi treatment may be partly responsible for the internal CO₂ partial pressure of only 15.9 Pa compared with 26.2 Pa for the 0.05 mM Pi leaves. The Cᵢ of the low Pi leaves is similar to that reported for low Pi spinach leaves (6), but the Cᵢ of the high Pi leaves is low for a C₃ plant. Cᵢ values ranged from 20 to 22.2 Pa for well-watered soybean leaves (7, 8), and 18.4 to 30.5 Pa for well-watered cotton leaves (21), while maize maintained a rather constant Cᵢ of 9.5 Pa across a variety of nutritional treatments (31). These low Cᵢ values for soybean are consistent with the relatively low stomatal conductance, high photosynthetic rate, and the high carboxylation efficiency observed here (Table I). If the metabolic machinery were capable of rapid photosynthesis, yet stomata were somewhat closed, as indicated by low stomatal conductance, Cᵢ might well be drawn down to a lower level than for other C₃ plants, as was observed in the high Pi treatment.

Reduced carboxylation efficiency may be the net result of direct effects of low Pi nutrition on the amount and/or activity of Rubisco or on the rate at which Calvin cycle intermediates are regenerated. Some workers have found that phosphorylation of Rubisco may enhance its activity (18), that Pi may enhance Rubisco activity (20), or that metabolite pools decrease under Pi deficiency (6). Under low Pi conditions, Pi may be more limiting for the regeneration of metabolites in the Calvin cycle than for the phosphorylation of enzymes. Insufficient regeneration of RuBP and reduced specific activity of Rubisco could lead to the observed reduction in photosynthesis and carboxylation efficiency. Rubisco content per area decreased 24% and Rubisco activity per area decreased 33%. Low Pi spinach also had lower Rubisco activity on an area basis and the enzyme had a lower level of activation (6). The reduction in Rubisco content or specific activity was less than the decrease in A (65%) and the increase in the CO₂ compensation point (71%) in response to low Pi. Although the change in A and CO₂ compensation point need not be proportional to the change in Rubisco content or activity, the large deviation from proportionality suggests the involvement of additional factors, e.g. RuBP regeneration.

It is unclear if an increase in the CO₂ compensation point is a general response to stress. The CO₂ compensation point can increase with environmental conditions such as low N (14), N source (25), high leaf temperature (3), high O₂ concentration, seasonal variation, and leaf age (3). Whereas the increase in the CO₂ compensation point was about 1.5 Pa for N-stressed barley (14), we observed an increase of 5.0 Pa for Pi-stressed soybean leaves, yet Pi-stressed spinach showed no shift in the compensation point (6). The increase in the CO₂ compensation point might in part explain the decreased A of the low Pi treatment: a greater amount of CO₂ must be fixed just to compensate for the CO₂ evolution.

The increase in the CO₂ compensation point may suggest elevated respiration in the low Pi treatment. Elevated respiration may be due to increased photorespiration, increased dark respiration in the light, and/or increased activity of the alternate pathway. It is unlikely that photorespiration is elevated in relation to carbon fixation because this implies a decrease in enzyme specificity for CO₂ relative to O₂, for which there is little evidence; however, the observed change in the specific activity of Rubisco (Table II) raises this possibility. Pi is itself a substrate for mitochondrial respiration, so it seems unlikely that dark respiration would be stimulated in the low Pi tissue. However, the alternate pathway of respiration is active in soybean leaves (23), and as it is largely nonphosphorylating, low Pi may have less effect upon its ability to operate.

Soybean responds to Pi stress with decreased A, a reduction in light saturation point, reduced A at light saturation, lower quantum efficiency, reduced carboxylation efficiency, and elevated CO₂ compensation point. Decreased Chl and soluble protein concentration, decreased Rubisco, and reduced specific activity of remaining Rubisco account in part for the decrease in efficiency of the transfer of light energy into reduced carbon. As the magnitude of these changes is only about 50% of the decrease in A and less than 50% of the increase in the CO₂ compensation point, further investigation of the adaptive response of leaf metabolism to low Pi conditions seems warranted. This report documents the paraheliotropic pattern of leaf movement and photosynthetic responses of CO₂ assimilation under low Pi nutrition, but the cause of the large increase in the CO₂ compensation point remains to be elucidated.

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LITERATURE CITED


