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Phosphorus Accumulation and Utilization Efficiency in Soybean Plant under Atmospheric CO_2 Enrichment

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Soybean plants(*Glycine max* [L.] merr.) inoculated with *Bradyrhizobium japonicum* MN110 were grown in growth chambers under 400 or 800 $\mu l \cdot l^{-1}$ atmospheric CO_2 and harvested at 25, 28, 32, and 35 DAT to examine the effect of CO_2 enrichment on phosphorus accumulation, uptake, and utilization efficiency during vegetative growth. Phosphorus concentration in leaf was lower in high CO_2 plant by 47% at 25 DAT and 34% at 35 DAT than those in the control plant but phosphorus concentrations in stem, root and nodule were not affected by CO_2 enrichment. Total phosphorus accumulation increased 3.9-fold in high CO_2 plant and 3.2-fold in the control plant between 25 and 35 DAT. Elevated CO_2 caused a decrease in the whole plant phosphorus concentration by 35%, which was due almost entirely to a decrease in the phosphorus concentration of leaves. CO_2 enrichment increased phosphorus utilization efficiency in the whole plant by 70% during the experimental period. Plants exposed to high CO_2 had larger root systems than under ambient CO_2, but high CO_2 plants had lower P-uptake efficiency. Averaged over four harvests, plants at high CO_2 had 38% larger root mass that was more than offset the 20% lower efficiency of P-uptake and accounted for increased phosphorus accumulation by high CO_2 plant. These results suggest that the reduced phosphorus concentration in soybean plant under CO_2 enrichment may be an acclimation response to high CO_2 concentration or enhanced starch accumulation, resulting in the plants to have a lower phosphorus requirement on a unit dry weight basis or a high phosphorus utilization efficiency under these conditions.

Key words: *CO_2 enrichment, soybean, P-accumulation, P-uptake, P-utilization efficiency.*

There are sufficient evidences that the level of atmospheric CO_2 will have a major impact on the growth of plants, irrespective of any changes in climate that may accompany the rise in CO_2.[1,2] The acceptance of this concept has led to the investigation of plant responses to nutrition at different levels of atmospheric CO_2.[3] The CO_2 enrichment will probably have a big impact on carbon assimilation by plants and also may result in significant increases in growth and yield. Findings on the acclimated growth response are sometimes contradictory. For example, Mousseau and Enoch[4] found a large fraction of plant dry matters in roots at enriched CO_2, whereas Tolley and Strain[5] found the root fraction to be smaller under the same condition. Koch *et al.*[6] reported that no differences in dry matter allocation were found between different CO_2 treatments.

The increase in productivity occurs when nutrition availability is high. Phosphorus limits crop productivity more frequently than any other nutrient except nitrogen.[7] Plants fail to respond to high CO_2 when phosphorus is low, probably because phosphorus plays an important role in the photosynthetic carbon metabolism of leaves and insufficient phosphorus is not available to maintain maximum photosynthetic activity at high CO_2.[8,9] The critical phosphorus concentration for *P. radiata* was higher at elevated CO_2[10], and the phosphorus concentration of the whole soybean plant for maximum productivity was 0.4 and 0.8% at ambient and elevated CO_2, respectively.[11]

Little attention has been given to the phosphorus uptake and utilization efficiency under CO_2 enrichment. Hence, the purpose of this experiment was to examine the effect of CO_2 enrichment on growth characteristics, phosphorus uptake, and utilization efficiency in whole nitrogen fixing soybean plant during vegetative growth.

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Abbreviations: DAT, days after transplanting; DW, dry weight.

Materials and Methods

Plant Culture. Soybean (*Glycine max* [L.] Merr.) plants inoculated with *Bradyrhizobium japonicum* MN 110 were grown in chambers with either 400 or 800 $\mu l \cdot l^{-1}$ atmospheric CO_2. Carbon dioxide concentrations were monitored using an infrared gas analyzer and maintained at the desired concentrations by either scrubbing the recycled air or injecting pure CO_2 into the chambers.[12] High pressure sodium vapor and metal halide lamps provided a photosynthetic photon flux density (PPFD) of approximately 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The temperatures of the chambers were controlled at 26/22°C during the 9 h/15 h light/dark cycle. The relative humidities for the light and dark periods were approximately 70 and 100%, respectively. Plants received 3 h interruption of dark period with photomorphogenic irradiance to suppress flowering during the experimental period. The composition of nutrient solution was the same as described by Israel and Jackson.[13] Roots of seedlings (3 days old) were dipped into the fresh inoculum (10^9 colony forming units · ml^{-1}) of *Bradyrhizobium japonicum* MN 110 just before transplanting into 25.4 cm pots filled with moist Perlite. Immediately after transplanting, 0.5 m*l* of inoculum was applied at the base of each seedling. From 1 to 4 DAT each pot was irrigated daily with sufficient deionized water to keep the Perlite moist; from 5 to 12 DAT, each pot was supplied daily with 0.5 *l* of deionized water at 0800 h and with 0.5 *l* deionized water followed by 0.25 *l* of the nutrient solution at 1400 h. After 13 DAT, water applications were increased to 1.5 *l* per pot and the amount of nutrient solution applied at 1400 h was increased to 0.5 *l*. Plants were harvested at 25, 28, 32, and 35 DAT and separated into leaflet, stem and petiole, root, and nodule. After measuring the leaf area, the plant material was dried, weighed, and ground to pass an 1 mm screen.

Growth and Phosphorus Analysis. Leaf area was measured photometrically using leaf area meter (Model LI-3000, LICOR, USA). Specific leaf weight and leaf area ratio were calculated on the basis of leaf dry weight · leaf $area^{-1}$ (mg · cm^{-1}) and leaf area · plant dry $mass^{-1}$ ($cm^2 \cdot g^{-1}$), respectively. For determination of total phosphorus concentration, tissue samples (100-200 mg) were digested according to Kjeldahl's procedure that included a salicylic acid predigestion step and employed a Cu-Zr catalyst.[14] Appropriate aliquots were analyzed for phosphorus through the ammonium-molybdate method of Murphy and Riley.[15] Total phosphorus absorbed per gram of root dry weight at each harvest was used as an indicator of the relative index of P-uptake efficiency. Phosphorus utilization efficiency was calculated by dividing the total dry matter by the whole plant phosphorus concentration.[16]

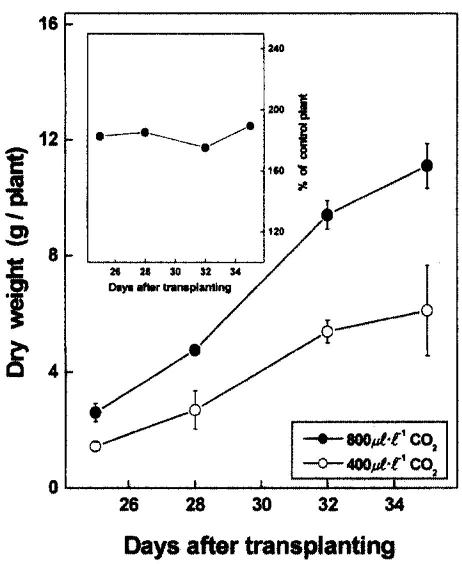

Fig. 1. Effect of atmospheric CO_2 enrichment on dry mass accumulation in soybean plant during the vegetative growth. Small bar at each point represents standard deviation (n = 3). The figure in the small box represents the relative dry weight of plant grown under 800 μ*l* · l^{-1} CO_2 to the control plant (400 μ*l* · l^{-1} CO_2).

Results and Discussion

Aerial CO_2 enrichment had a pronounced impact on DW accumulation by the vegetative soybean plant. Both CO_2-treated and the control plants showed 4.3-fold increase from 25 to 35 DAT, but DW of high CO_2 plants was 78% greater than the control plant during the 10 day experimental period (Fig. 1).

Carbon dioxide enrichment resulted in increased total leaf area and leaf DW accumulation (Fig. 2), but the degree of increase in leaf DW accumulation was higher than that in the total leaf area from 25 to 35 DAT. When averaged over all harvests, CO_2 enrichment increased specific leaf weight by 24%, but decreased leaf area ratio by 40% (Fig. 2). The enhancement of dry matter accumulation by elevated CO_2 was associated with the plant leaf area and the increase in specific leaf weight. Increased specific leaf weight caused by atmospheric CO_2 enrichment has been shown to be associated primarily with increased starch accumulation.[17] Increased whole plant leaf area and specific leaf weight indicate that atmospheric CO_2 enrichment increased the leaf DW by enhancing both the

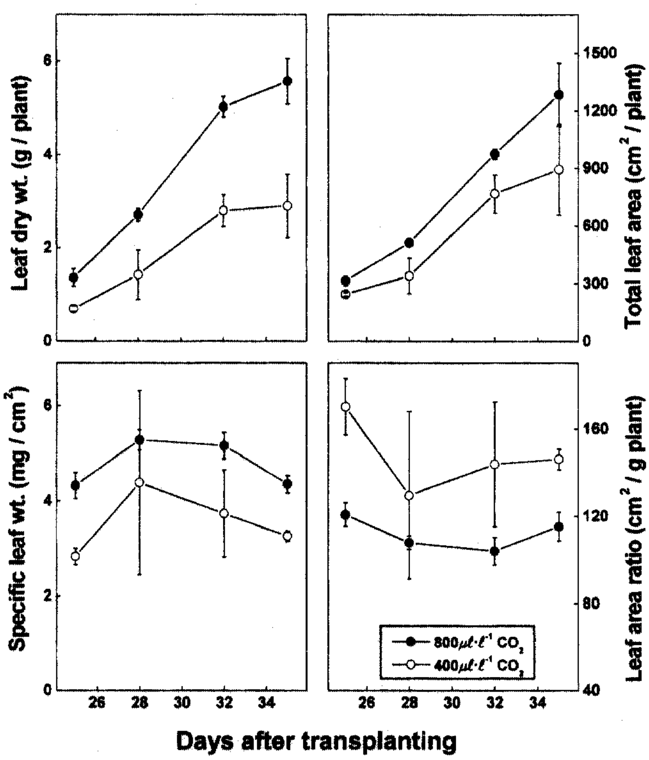

Fig. 2. Effect of atmospheric CO_2 enrichment on leaf dry mass, total leaf area, specific leaf weight and leaf area ratio in soybean plant during the vegetative growth. Small bar at each point represents standard deviation (n = 3).

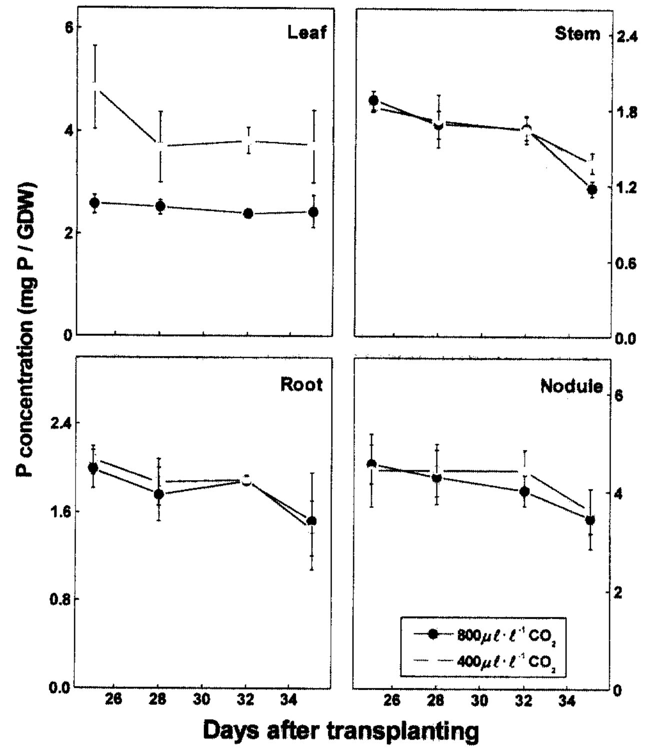

Fig. 3. **Effect of atmospheric CO_2 enrichment on phosphorus concentration in leaf, stem, root and nodule tissue of soybean plant during the vegetative growth.** Small bar at each point represents standard deviation (n = 3).

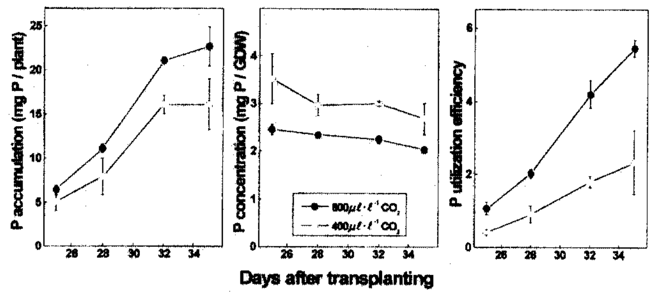

Fig. 4. Effect of atmospheric CO_2 enrichment on phosphorus accumulation, concentration and utilization efficiency in soybean plant during the vegetative growth. Small bar at each point represents standard deviation (n = 3).

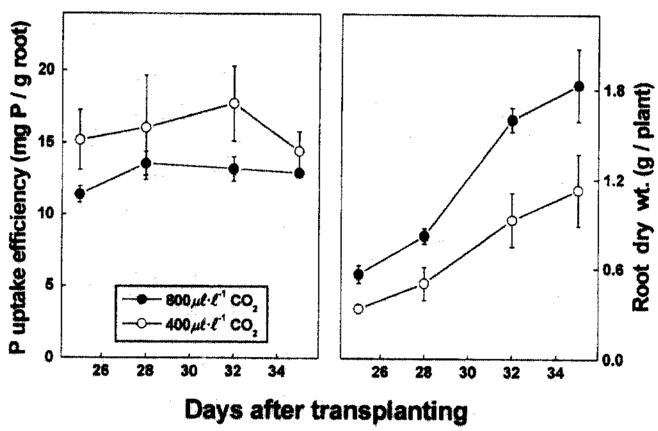

Fig. 5. Effect of atmospheric CO_2 enrichment on P-uptake efficiency and root dry weight in soybean plant during the vegetative growth. Small bar at each point represents standard deviation (n = 3).

synthesis of structural components and the accumulation of starch.[18] The higher increase in leaf dry mass and the higher specific leaf weight are likely due to the starch accumulation in leaves under CO_2 enrichment. In a soybean study, when CO_2 level was elevated from 330 to 800 $\mu l \cdot l^{-1}$, an increase in specific leaf weight was also accompanied by an increase in leaf starch concentrations.[19] Wheeler *et al.*[17] reported that excess starch accumulation in leaves occurred under CO_2 enrichment, and extremely high starch concentrations in source leaves may inhibit the CO_2 assimilation and result in a lower photosynthetic rate.[20]

Phosphorus concentration in stem, root, and nodule were stable in both CO_2 treatments during experimental period and were not affected by CO_2 enrichment. Phosphorus concentrations in leaf were lower in high CO_2 plant by 47% at 25 DAT and 34% at 35 DAT than those of the control plant. It is likely that phosphorus concentration in the leaf was diluted by the high starch accumulation under CO_2 enrichment so that less phosphorus appeared to be available to the tissue than actually was present. Cao and Tibbitts[21] reported that nutrient concentrations were related to starch concentrations only in a range of less than 14% starch level. However, even under 14% starch level, changes in starch concentrations can only partially accounted for the variation in nutrient concentrations. These results indicate that nutrient dilution by high starch concentration in tissue is not the only factor accounting for the reduced nutrient concentrations under CO_2 enrichment in certain plants such as soybean. Several studies on sugar beet[22] and wheat[23] showed that plant growth under high CO_2 did not improve with enhanced mineral nutrition.

Total phosphorus accumulation increased 3.9 fold in high CO_2 plant and 3.2-fold in the control plant between 25 and 35 DAT (Fig. 4). Elevated CO_2 caused a decrease in the whole plant phosphorus concentration by 35% (Fig. 4), which was due almost entirely to a decrease in the phosphorus concentration of leaves (Fig. 3). This effect was related, however, to a marked stimulation of phosphorus utilization efficiency (Fig. 4) in the accumulation of dry matter. Siddiqi and Glass[16] proposed that, since the internal concentration of an essential nutrient must be above a certain critical level for optimal plant growth, nutrient-utilization efficiency should be calculated by diving the whole plant biomass by the concentration of the nutrient in the biomass, thus their approach was use herein. Carbon dioxide enrichment increased phosphorus utilization efficiency in the whole plant by 70% during the experimental period (Fig. 4). The increase in utilization efficiency was associated with enhanced dry matter production (Fig. 1). Since atmospheric CO_2 enrichment reduced P-uptake efficiency of roots (Fig. 5), its stimulation of root growth apparently allowed nutrient uptake from a greater volume of the rooting medium.

Total P-uptake by plants is the product of total root dry mass and average uptake per unit of root mass (uptake efficiency). Plants exposed to high CO_2 had larger roots systems than ambient CO_2, but high CO_2 plants had lower P-uptake efficiency (Fig. 5). Averaged over four harvests, plants at high CO_2 had 38% larger root mass that was more than offset the 20% lower efficiency of P-uptake and accounted for increased phosphorus accumulation by high CO_2 plants. The results of this study suggest that the reduced phosphorus concentration in soybean plant under CO_2 enrichment may be an acclimation response to high CO_2 concentrations or enhanced starch accumulation, resulting in the plants to have a lower phosphorus requirement on a unit dry weight basis or a high phosphorus utilization efficiency under these conditions.

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