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Effect of Phosphorus Stress on Photosynthesis and Nitrogen Fixation of Soybean Plant under CO_2 Enrichment

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Abstract : The objective of this study was to examine the effect of phosphorus deficiency on nitrogen fixation and photosynthesis of nitrogen fixing soybean plant under CO_2 enrichment condition. The soybean plants(*Glycine max* [L.] Merr.) inoculated with *Bradyrhizobium japonicum* MN 110 were grown with P-stressed(0.05 mM-P) and control(1 mM-P) treatment under control(400 μl/L CO_2) and enrichment(800 μl/L CO_2) enviromental condition in the phytotron equipped with high density lamp(1000 $\mu Em^{-2}S^{-1}$) and 28/22℃ temperature cycle for 35 days after transplanting(DAT). At 35 DAT, phosphorus deficiency decreased total dry mass by 64% in CO_2 enrichment condition, and 51% in control CO_2 condition. Total leaf area was reduced significantly by phosphorus deficiency in control and enriched CO_2 condition but specific leaf weight was increased by P deficiency. Phosphorus deficiency significantly reduced photosynthetic rate(carbon exchange rate) and internal CO_2 concentration in leaf in both CO_2 treatments, but the degree of stress was more severe under CO_2 enrichment condition than under control CO_2 environmental condition. In phosphorus sufficient plants, CO_2 enrichment increased nodule fresh weight and total nitrogenase activity(acetylene reduction) of nodule by 30% and 41% respectively, but specific nitrogenase activity of nodule and nodule fresh weight was not affected by CO_2 enrichment in phosphorus deficient plant at 35 DAT. Total nitrogen concentrations in stem, root and nodule tissue were significantly higher in phosphorus sufficient plant grown under CO_2 enrichment, but nitrogen concentration in leaf was reduced by 30% under CO_2 enrichment. These results indicate that increasing CO_2 concentration does not affect plant growth under phosphorus deficient condition and phosphorus stress might inhibit carbohydrate utilization in whole plant and that CO_2 enrichment could not increase nodule formation and functioning under phosphorus deficient conditions and phosphorus has more important roles in nodule growth and functioning under CO_2 enrichment environments than under ambient condition.(Received January 31, 1997; accepted February 14, 1997)

Introduction

The global atmospheric CO_2 concetration is increasing. Most predictive models indicate that it is likely to double by the second half of the next century.[1] Increase in atmospheric CO_2 concentration are expected to increase the growth and yield of C_3 crop plant, primary through stimulation of photosynthetic activity.[2] Symbiotic N_2 fixation in legumes requires carbon substrates derived from photosynthate or from stored reserves.[3] The substrates provide a source of energy for N_2 fixation and acceptor molecules for transporting reduced N. The original source of such carbon compounds is primarily photosynthetic CO_2 reduction. Claims that photosynthate limits N_2 fixation in soybean were supported by long-term CO_2-enrichment studies in field grown plant.[4-6] Plant responses to increasing CO_2 will be governed to a large extent by other factors, *e.g.*, water and nutrient availability, which collectively are involved in defining plant growth potential.[7] Phosphorus limits crop productivity more frequently than any other nutrient except nitrogen, and has an important role in nodule growth and function as well as plant growth. An improvement in phosphorus status of several legumes including soybeans, has been reported to increase nitrogen concentration in plant tissues as well as overall plant growth.[8,9] There has been little research to determine how phosphorus stress influences plant physiological response to atmospheric CO_2 enrichment. Goudriaan and de Ruiter[10] compared the dry matter accumulation in lucene (*Medicago sativa* L.), fababean (*Vicia faba* L.), permmial ryegrass (*Lolium perenne* L.), and wheat (*Triticumaestivum* L.), when cultured under either adequate or severely deficient P conditions. Fababean was the only plant to exhibit a positive dry matter accumulation response to elevated CO_2 under P-limited conditions. The magnitude of the response, however, was reduced compared to that with adequate P, and CO_2 enrichment had no effect on seed yield. The purpose of this experiment was to examine the effect of P

Key words : CO_2 enrichment, phosphorus, nitrogen fixation, photosynthesis

nutrition on the plant growth, nitrogen fixation and photosynthesis of nitrogen fixing soybean plants under CO_2 enrichment condition during the vegetative growth.

Materials and Methods

Plant culture and inoculation

Soyben (*Glycine max* [L.] Merr.) plants were grown in the phytotron controlled 400(control) and 800(high) $\mu l/l$ CO_2 atmosphere. Carbon dioxide concentrations in chambers were monitored with infrared gas analyzer and maintained at desired concentrations by either scrubbing recycled air or injecting pure CO_2 into the chambers.[11] Plants were irradiated with high pressure sodium vapor and metal halide lamps, providing a photosynthetic photon flux density (PPFD) of approximately 1,000 μmol $m^{-2}s^{-1}$ at the average leaf canopy height. The temperature in all chambers were controlled at 28/22°C during the 9/15-h light/dark cycle. The relative humidities for the light and dark periods were approximately 70% and 100%, respectively. Throughout the course of experiment, the plants recevied 2h of low PPFD (50 μmol $m^{-2}s^{-1}$)from incandescent lamps midway through the dark period to repress floral induction. Nutrient solutions were prepared in tap water and the composition of nutrient solution was same as described by Israel and Jackson.[12] Nutrient solution used for the deficient treatment contained 0.05 mM-P and that for the control treatment contained 1.0 mM-P. Roots of seedlings (3 day old) were dipped in the fresh inoculum (10^9 colony forming units/l) of *Bradyhizobium Japonicum* MN 110 just before transplating. Immediately after transplanting, 0.5 ml of inoculum was applied at the base of each seedling. Other growth conditions were the same as deseribed by Israel and Jackson.[12] Plant material was harvested at the 28, 35 days after transplanting (DAT). Plants were separated into leaflet, stem plus petiole, root and nodule fractions. All plant materials were dried at 65°C for 72h, weighed and ground to pass through a 1 mm screen.

Photosynthesis measurement

Carbon Exchange Rate and Stomatal Resistance were measured simultaneously with a Li-Cor Model 6200 Portable Photosynthesis System.

Nitrogenase activity assay

Acetylene reduction assays were performed on the excised root systems.[13] Nodulated root systems were incubated for 30 min at 25°C in 1-L jars containing 0.1 atm. of acetylene. Ethylene and acetylene in the samples were separated and quantified with a Carle model 311 H gas chromatograph equipped with flame ionization detector and a column of Porapak N(182×0.32 cm).

Nitorgen determination

The total N concentration of tissue samples (100~200 mg) was determined by a Kjeldahl procedure that included a salicylic acid predigestion step and employed a copper-zirconium catalyst.[14] After alkalinization of digests, ammonia was steam-distilled into boric acid and quantified by titration with potassium biiodate.

Results and Discussion

The whole dry mass of the phosphorus deficient plant was significantly lower than that of phosphorus sufficient plant under both CO_2 treatments, but the degree of growth inhibition by phosphorus deficiency is more pronounced under high CO_2 treatment than under control CO_2 treatment(Table 1). The dry mass accumulation, at 35DAT, in leaf and nodule tissue was reduced by phosphorus deficiency by 68% and 82%, respectively, under high CO_2 treatment, and by 55% and 80%, respectively, under control CO_2 treatment, but the dry mass accumulation in root increased by 5% in phosphorus deficient plant under high CO_2 treatment. The level of phosphorus under which plants were grown affceted dry matter partitioning among plant parts (Table 2). The proportion of whole plant mass in nodule decreased by 62% and 58% but that in root increased by 45% and 46% in phosphorus deficient plant

Table 1. Effect of phosphorus deficiency on the plant dry mass under CO_2 enrichment condition during the vegetative growth.

DAT	P (mM)	CO_2 ($\mu l/l$)	Leaf	Stem	Root	Nodule	Total
			g/plant				
28	1.0	800	2.70 (0.13)	0.92 (0.02)	0.82 (0.05)	0.31 (0.01)	4.75 (0.18)
		400	1.42 (0.53)	0.53 (0.07)	0.50 (0.11)	0.24 (0.04)	2.69 (0.66)
	0.05	800	1.26 (0.10)	0.44 (0.09)	0.59 (0.15)	0.07 (0.02)	2.36 (0.19)
		400	0.96 (0.15)	0.41 (0.05)	0.49 (0.15)	0.07 (0.01)	1.93 (0.36)
35	1.0	800	5.57 (0.36)	2.86 (0.14)	1.83 (0.18)	0.85 (0.08)	11.11 (0.55)
		400	2.89 (0.48)	1.58 (0.37)	1.13 (0.17)	0.52 (0.10)	6.11 (1.12)
	0.05	800	1.83 (0.34)	0.97 (0.08)	1.10 (0.06)	0.16 (0.03)	4.06 (0.50)
		400	1.29 (0.16)	0.70 (0.09)	0.87 (0.14)	0.10 (0.02)	2.96 (0.41)

( ) represents standard deviation (n=3).

Table 2. Effect of phosphours deficiency on the dry mass distribution(%) in leaf, stem, root and nodule of soybean plant during the vegetative growth.

DAT	P (mM)	CO_2 ($\mu l/l$)	Leaf	Stem	Root	Nodule
28	1.0	800	56.78 (0.58)	19.30 (0.65)	17.32 (0.41)	6.60 (0.13)
		400	51.70 (7.02)	20.00 (2.03)	19.37 (4.40)	8.93 (0.58)
	0.05	800	53.70 (5.16)	18.38 (1.70)	24.83 (4.00)	3.09 (0.27)
		400	50.01 (1.07)	21.20 (0.92)	24.98 (2.03)	3.81 (0.18)
35	1.0	800	50.07 (0.76)	25.77 (0.11)	16.47 (0.97)	7.69 (1.10)
		400	47.40 (0.91)	25.43 (1.60)	18.59 (1.70)	8.58 (0.23)
	0.05	800	44.67 (2.72)	24.13 (1.22)	27.32 (2.17)	3.87 (0.42)
		400	43.56 (0.77)	23.66 (0.37)	29.36 (0.70)	3.43 (0.45)

( ) represents standard deviation (n=3).

Table 3. Effect of phosphorus deficiency on the total leaf area, specific leaf weight and nodule/whole plant ratio under CO_2 enrichment condition during the vegetative growth.

DAT	P (mM)	CO_2 ($\mu l/l$)	Total leaf area	Specific leaf weight	Nodule/whole plant
			cm^2/plant	mg/cm^2	
28	1.0	800	511.3 (4.89)	5.28 (0.15)	0.0659 (0.0013)
		400	339.3 (62.89)	4.38 (1.30)	0.0881 (0.0058)
	0.05	800	256.0 (79.33)	5.49 (1.50)	0.0311 (0.0027)
		400	216.0 (15.33)	4.45 (0.30)	0.0379 (0.0018)
35	1.0	800	1283.0 (122.00)	4.35 (0.12)	0.0762 (0.0110)
		400	892.7 (159.78)	3.25 (0.08)	0.0856 (0.0023)
	0.05	800	383.3 (60.44)	4.75 (0.23)	0.0394 (0.0042)
		400	305.3 (30.44)	4.21 (0.21)	0.0349 (0.0045)

( ) represents standard deviation (n=3).

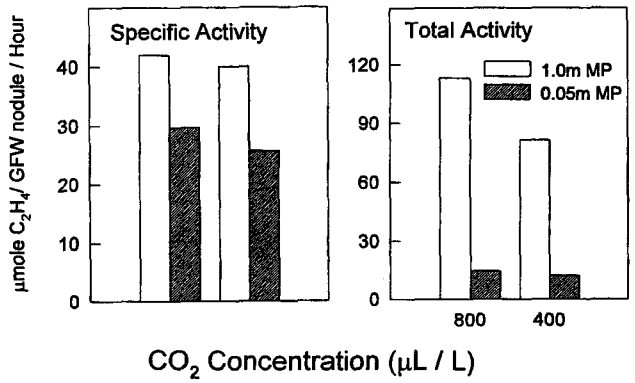

Fig. 1. Effect of phosphorus deficiency on the specific and total nitrogenase activity of soybean nodules under CO_2 enrichment at 35DAT.

under control and high CO_2 treatment, respectively, at 35DAT. The effects of phosphorus deficiency on total leaf area, specific leaf weight and nodule/whole plant ratio are presented at Table 3. Increasing the CO_2 level at which plant were grown affected total leaf area of soybean plant significantly under both phosphorus treatments. The proportional increase in total leaf area in response to CO_2 enrichment were greater in phosphorus sufficient plant than in phosphorus deficient plant. Cure *et al.*[15] reported that main effect of high CO_2 was an increase in the development rates of the plant leaf canopy, which involved both leaves on the main stem and leaves on lateral branches and leaf initiation and expansion have been shown to be regulated by the rate of nitrogen assimilation and the delivery of reduced nitrogen to the shoot meristems.

The phosphorus deficiency increased specific leaf weight significantly in both CO_2 treatments. In phosphorus sufficient plant nodule/whole plant ratio ranged 0.0659~0.0762 under high CO_2 treatment and 0.0881~0.0856 under control CO_2 treatment, but that in phosphorus deficient plant was around 0.03~0.04 under both CO_2 treatments. This indicate that the degree of restriction is more severe in nodule growth and development than host-plant growth when the host plant was under phosphorus deficiency, and whole plant growth is faster than nodule growth under high CO_2 atmospheric conditions.

Specific and total nitrogenase activity of nodule at 35 DAT were illustrated in Fig. 1. CO_2 enrichment increased significantly total nitrogenase activity in P-sufficient plant but the specific nitrogenase activity was not affected by CO_2 enrichment in P-sufficient plant. These represent that CO_2 enrichment activate nodule growth more than nodule functioning at vegetative growth(Table 1., Fig. 1). Herrige and Pate[16] reported that 30% of the carbon supplied of pea plant was used to nodule growth and 20% of that was used to nitrogen fixation in the nodule under ambient CO_2 condition. Nitrogen concentration in leaf and nodule was reduced by CO_2 enrichment in P-sufficient plant, and that in not tissue was not affected by CO_2 enrichment(Fig. 2) but the nitrogen accumulation in all tissues increased significantly by CO_2 enrichment in P-sufficient plant(Table 4). It seems that the more rapid growth rate and increased dry matter production of plants in high CO_2 treatments presumably result in dilution of nitrogen in the leaf and nodule tissue in vegetative stage under high CO_2 condition(Table 1. 4., Fig 2). The enhancing effect of enriched CO_2 concentration on carbon exchange rate and internal CO_2 concentration in soybean leaf was more pronounced in phosphorus sufficient plant than in phosphorus deficient plant (Fig 3). Exposure of phosphorus sufficient plant to high CO_2 environment increased rate of net photosynthesis by 6% and internal CO_2 concentration by 3%. Maintenance of elevated pho-

Table 4. Effect of phosphorus deficiency on the nitrogen accumulation in soybean plant under CO_2 enrichment condition at 35 days after transplanting.

DAT	P (mM)	CO_2 ($\mu l/l$)	Leaf	Stem	Root	Nodule	Total
			mg/plant				
35	1.0	800	96.33 (18.1)	23.01 (4.05)	23.03 (2.81)	29.01 (7.70)	171.4 (32.5)
		400	70.02 (22.8)	12.27 (2.86)	16.87 (1.13)	15.48 (7.34)	114.6 (32.9)
	0.05	800	35.56 (4.90)	8.06 (1.62)	15.11 (2.86)	4.46 (0.71)	63.2 (10.0)
		400	40.72 (5.84)	6.98 (0.98)	14.67 (1.22)	4.82 (1.22)	67.2 (8.75)

( ) represents standard deviation (n=3).

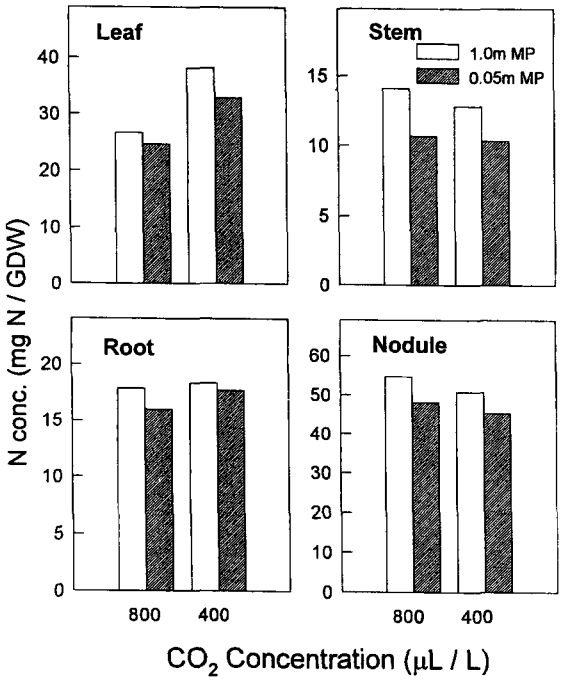

Fig. 2. Effect of phosphorus deficiency on the nitrogen concentration in leaf, stem, root and nodule tissue in soybean plant under CO_2 enrichment at 35DAT.

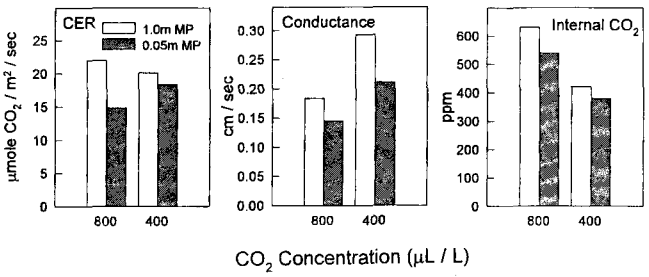

Fig. 3. Effect of phosphorus deficiency on the carbon exchange rate, conductance and internal CO_2 concentration in leaf of soybean plant under CO_2 enrichment at 28DAT.

tosynthetic rate seems to depend on the activity of growth sinks and associate with ability to utilize the increased supply of photosynthate. The degree of increase in photosynthesis is less than expectation from growth modelling. It might be seemed that high starch accumulation and low utilization efficiency of starch in leaf and/or the change of allocation of N in the chloroplast inhibit photosynthesis at high CO_2. Ryle *et al.*[17] reported that elevating CO_2 concentration promotes the accumulation of fixed carbon but has little or no known effect on its consumption of fixed carbon, with the corollary that the metabolic balance source and sink for assimilate is likely to be perturbed, and, in extreme cases, accumulation of starch can damage the chloroplast. A large proportion of leaf N is associated with proteins located in the chloroplast. The majority of these proteins are involved in photosynthesis, with Rubisco accounting for about 25% of the total leaf N, and proteins associated with photosynthetic electron transport for a futher 25%.[18] Allocation of N to these pools may be reduced at high CO_2 due to change in the efficiency of photosynthesis. The results of this experiment indicates that increasing CO_2 concentration does not affect plant growth, nitrogen fixation and photosynthesis significantly under phosphorus deficiency condition. Dudal[19] reported that approximately 20% of the earth's land mass was sufficiently low levels of nutrients to cause some kind of stress to plant. Change in nutrient uptake, remobilization and efficiency of utilization in the plant at elevated CO_2 will be characterized to develop fertilizer management in agricultural system and nutrient cycling in natural ecosystems under increasing atmospheric CO_2 concentration.

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대기 CO_2 상승시 인산공급이 식물체의 광합성 및 질소고정에 미치는 영향

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초록 : 대기 CO_2 상승시 인산공급이 대두식물체의 광합성 및 질소고정에 미치는 영향을 살펴보고저 *Bradyrhizobium japonicum* MN110을 접종한 대두 식물체를 인산결핍구(0.05 m-P) 및 대조구(1.0 mM-P)를 처리하여 정상 CO_2(400 μl/L)와 상승 CO_2(800 μl/L)의 조건인 phytotron에서 35일 동안 재배하였다. 인산결핍은 건물량을 정상 CO_2 처리시 51%, 상승 CO_2 처리시 64%를 감소시켰고 총 엽면적도 유의성있게 감소시켰으나 specific leaf weight는 증가시켰다. 광합성 속도 및 잎내부의 CO_2농도는 인산결핍구에서 감소하였고 그 정도는 CO_2 상승처리시 더욱 컸으며 질소고정능과 근류생체량도 인산결핍시 감소하였으나 잎의 질소농도는 인산대조구가 30% 감소하였다. 이러한 결과는 CO_2 상승조건에서도 인산이 결핍될 경우 식물생육을 촉진시키지 못하며 인산은 정상 CO_2 농도시 보다 CO_2 상승조건에서 근류의 성장과 작용에 더욱 중요한 역할을 하는것으로 유추된다.

찾는말 : CO_2 enrichment, 인, 질소고정, 광합성