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Ureide Distribution in Nitrogen Fixing Soybean Plant under External Phosphorus Limitation

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Abstract : Soybean plants inoculated *Bradyrhizobium japonicum* MN 110 were grown in outdoor perlite pots with nitrogen free nutrient solution containing 1.0 mM-P(control) and 0.05 mM-P(stress) and harvested at 28, 35, 42 and 49 days after transplanting (DAT) to examine the effect of phosphorus deficiency on ureide concentration of and distribution to diffrent plant organ in nitrogen fixing soybean plant during the vegetative growth. Total dry mass of control plants increased 8.9 fold and that of phosphorus deficient plant increased 2.7 fold during the experimental period. Phosphorus deficiency reduced total phosphorus and nitrogen accumulation by 80%, 40% respectively, at 28 DAT and 93%, 84%, respectively, at 49 DAT. Nitrogen concentration was reduced by phosphorus deficiency in all tissues with leaf and stem tissues affected to a greater degree than nodule and root tissues at every sampling date. Phosphorus deficiency significantly reduced soluble reduced-N and ureide-N concentration in leaf and stem but did not affect those in root. The proportion of soluble reduced-N in leaf was reduced from 60% to 50% but increased from 10% to 20% in the roots. The proportion of ureide-N in leaf of control plants was higher than that in phosphorus deficient plants, whereas, roots of phosphorus deficient plants contained a higher propotion of ureide-N than those of control plants. These indicated that phosphorus deficiency not only inhibit nitrogen fixation of nodules but also restrict the translocation of fixed nitrogen out of the root system into the xylem.(Received April 4, 1997; accepted May 2, 1997)

Introduction

The source of nitrogen available to soybean plants has a profound effect on the forms of nitrogen translocated in the xylem.[1,2] When soybean plants are solely dependent on symbiotic N_2 fixation the ureides, allantoin and allantoic acid, contain approximately 80% of the total nitrogen in xylem sap.[1] On the other hand, asparagine and nitrate each contains approximately 40% of the total nitrogen in xylem sap of soybean plants solely dependent on nitrate. In terms of the overall carbon economy for transport of nitrogen, ureides which have a 1 : 1, C : N ratio may be more energy efficient than asparagine and glutamine which have 2 : 1 and 2.5 : 1, C : N ratio, respectively.[2] The phosphorus deficiency affects not only NO_3 uptake but also nitrogen partitioning into soluble and insoluble-N and distribution of these among whole plants dependent on nitrate. Long term phosphorus deficiency resulted in decreased total N concentrations in shoot organs.[3,4] Phosphorus deprivation for 20 days decreased the nitrate uptake rate, increased accumulatation of absorbed nitrate in the root, increased soluble reduced-N concentrations in all organs, increased asparagine accumulation in roots and stems, increased arginine accumulation in leaves, and decreased the insoluble reduced-N and total N concentrations in all organs. The different responses of shoot N concentration in nitrate supplied soybean plants to long-term exposure to suboptimal external P and to P deprivation may be related to the severity of the P deficiency, which was extreme after 20 days of P deprivation.[5] Phosphorus deficiency decreased nodule growth and nitrogen fixing activitity as well as plant growth.[6] But there has been little reserch to determine how phosphorus stress influence nitrogen partitioning and ureide distribution in nitrogen fixing soybean plant. Soluble reduced-N concentrations in tissues of N_2-fixing soybean plants at beginning seed fill were low under P-deficiency conditions and increased with improvement in P nutrition.[4] Hence, the objective of this study was to examine the effect of phosphorus deficiency on ureide concentration of and distribution to different plant organ in nitrogen fixing soybean plant during the vegetative growth.

Materials and Methods

Plant culture

Soybean seeds [*Glycine max.* [L.] Merr. 'Ransom']

Key words : Nitrogen Fixation, P-stress, Ureide

were germinted in germination papers saturated with 0.5 mM $CaSO_4$ in an incubator maintained at 30℃ and 95% RH for 72h. Seedlings were transplanted into 6L pots containing Perlite amended with 300 g of crushed oyster shells to control rhizosphere acidification.[7] Root of seedlings were dipped into a suspension of an early stationary phase culture of *Bradyrhizobium japonicum* MN 110 (10^9 colony forming units/mL) in galactose-arabinose-glutamate medium[8] just before transplanting. After transplanting, 0.5 mL of the same culture was applied to the Perlite at the base of each seedling. From 1 to 4 days after transplanting (DAT) each pot was irrigated with tap water; from 5 to 12 DAT, pots were supplied at 0800 and 1400 h with 500 mL of tap water followed by 250 mL of appropriate nutrient solution; and from 13 DAT to the last sampling date, the tap water irrigation was increased to 1.5 L and 500 mL of appropriate nutrient solution was supplied only after the 1400h irrigation. Nutrient solutions wtihout N were prepared in tap water as described by McClure and Israel[1] except that KH_2PO_4 was the sole source of P. The P-deficiency treatment solution contained 0.05 mM P and the control treatment solution contained 1.0 mM P. Plants were harvested at 28, 35, 42 and 49 days after transplanting and separated into leaves, stems, roots and nodules. Plant tissues were rapidly frozen in liquid N_2 immediately after sampling and placed on dry ice until transfer to a freeze dryer. After freeze drying, the material was weighed and ground to pass through a 1-mm screen.

Total nitrogen and phosphorus determination

Total nitrogen was determined by a Kjeldahl procedure that employed a copper-zirconium catalyst[9] and a salicylic acid predigestion step.[10] After alkalinization of the digests, ammonia was steam-distilled into boric acid and quantified by titration with potassium biiodate. Appropriate aliquots of diluted kjeldahl digest were analyzed for total phosphorus by the ammonium molybdate method of Murphy and Riley.[11]

Fractionation of nitrogen fractions

For determination of slouble-N fractions in leaves, stems, and roots samples (200 mg) were placed in screw cap test tubes containing 10 mL of 8.6 M ethanol in 0.05 M phosphate buffer, pH 7.0. After mixing, tubes were heated in an 80℃ water bath for 5 min. with shaking. After heating, the contents of the tubes were thoroughly mixed with a vortex mixer and centrifuged at 1600×g for 15 min. The supernatant fluid was transferred to other tubes and aliquots removed for ureide and soluble reduced-N determination. Soluble reduced-N in the extracts was determined by evaporating 5-mL aliquots to dryness in digestion tubes at 97℃ with aeration. The residue was digested and the ammonia quantified as described for the total N determination.

Ureide assay

Ureide-N were analyzed by the method of Young and Conway,[12] which involves degradation of allantion and allantoic acid to urea and glyoxylic acid and colorimetric determination of glyoxylic acid after derivatization with phenylhydrazine.

Results and Discussion

Total dry mass of control plants increased 8.9 fold and that of phosphorus deficient plant increased 2.7 fold during the experimental period (Fig. 1). Phosphorus deficiency reduced total phosphorus and nitrogen accumula-

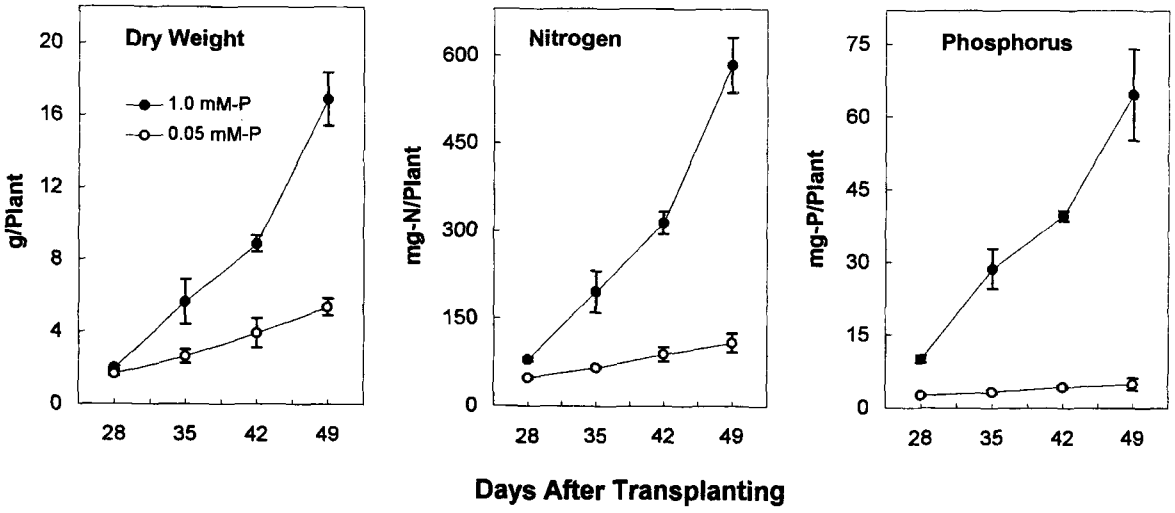

Fig. 1. Effect of external-P supply on dry weight, nitrogen and phosphorus accumulation in N_2-fixing soybean plants. Small bar at each point represents standard deviation (n=3).

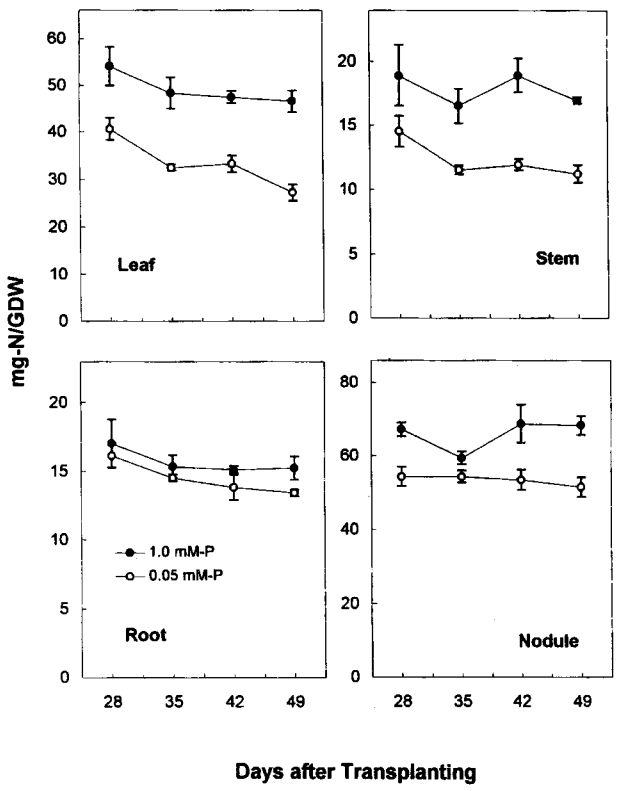

Fig. 2. Effect of external-P supply on nitrogen concentration in leaf stem, root and nodule tissues in N_2-fixing soybean plants. Small bar at each point represents standard deviation (n=3).

tion in whole plant by 80%, 40%, respectively, at 28 DAT and 93%, 84%, respectively, at 49 DAT. Nitrogen concentration in nodules were relatively constant in each treatment at 64 mg-N/gram dry weight(GDW) in nodules of control plants, 52 mg-N/GDW in nodules of phosphorus deficient plant, and these values were higher than those in any other tissues. The reduction in nitrogen concentration by phosphorus deficiency in all tissues was signifcant with leaf(33%) and stem(31%) tissues affected to a greater degree than nodule(19%) and root(8%) tissues during the experimental period (Fig. 2).

Reduction of nitrogen concentration and accumulation resulted form impairing nitrogen fixing activity of nodules and low nodule mass by phosphorus dificiency. Sa and Israel[13] reported that specific nitrogenase activity was decreased an average of 28% by continuous phosphorus deficiency and a significant increase in specific nitrogenase activity occured 2 days after removal of the external phosphorus limitation. Under the similar growth conditions as this experiment nodule growth was inhibited by 80% under the phosphorus stress.[14]

The soluble reduced-N concentration in leaf tissue was relatively constant from 28 to 49 DAT, at 7.1 mg-N/GDW for control plants and 4.6 mg-N/GDW for phosphorus deficient plants (Fig. 3). Phosphorus deficiency significantly decreased soluble reduced-N concentration

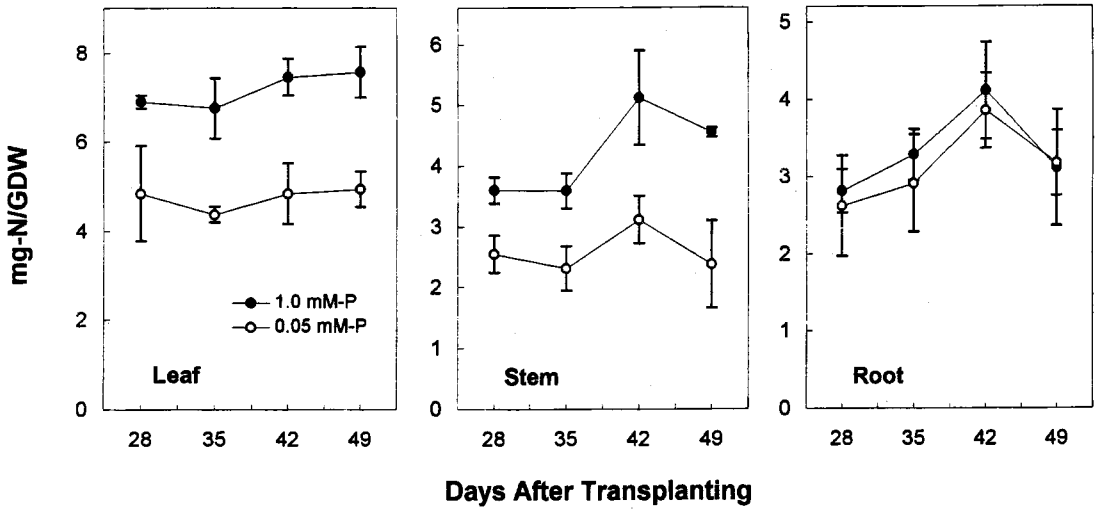

Fig. 3. Effect of external-P supply on soluble reduced-N concentration in leaf, stem and root tissues in N_2-fixing soybean plants. Small bar at each point represents standard deviation (n=3).

Table 1. Effect of external phosphorus supply on soluble reduced-N distribution to leaf, stem and root tissues of N_2-fixing soybean plants.

DAT	Leaf		Stem		Root	
	1.0 mM-P	0.05 mM-P	1.0 mM-P	0.05 mM-P	1.0 mM-P	0.05 mM-P
	%					
28	65.8(1.02)	52.9(4.54)	20.4(2.00)	27.2(3.42)	13.8(2.99)	19.9(5.22)
35	65.7(3.18)	56.1(5.39)	22.8(5.32)	23.8(5.38)	11.5(3.43)	20.1(4.53)
42	52.8(2.18)	37.2(4.76)	38.4(3.24)	32.4(7.75)	8.7(2.18)	30.4(9.09)
49	55.8(0.76)	54.7(1.59)	36.4(1.37)	20.5(2.53)	7.8(1.21)	24.8(3.88)

( ) represents standard deviation (n=3).

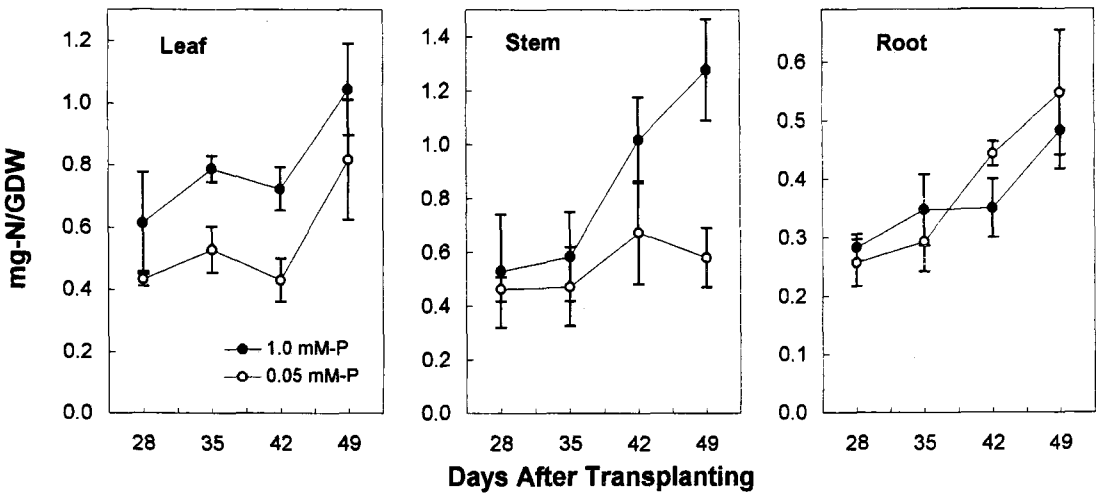

Fig. 4. Effect of external-P supply on ureide-N concentration in leaf, stem and root tissues in N_2-fixing soybean plants. Small bar at each point represents standard deviation (n=3).

Table 2. Effect of external phosphorus supply on ureide-N distribution to leaf, stem and root tissues of N_2-fixing soybean plants.

DAT	Leaf		Stem		Root	
	1.0 mM-P	0.05 mM-P	1.0 mM-P	0.05 mM-P	1.0 mM-P	0.05 mM-P
	%					
28	73.0(2.14)	62.3(6.75)	14.0(1.54)	15.9(0.34)	13.0(1.13)	21.8(6.92)
35	69.3(3.44)	59.9(4.10)	17.4(0.91)	15.0(1.68)	13.3(3.15)	25.1(2.43)
42	65.0(2.36)	50.7(6.42)	23.1(3.03)	18.3(3.26)	11.9(0.69)	31.0(4.65)
49	69.3(3.22)	59.7(5.03)	22.2(2.02)	14.8(3.58)	8.5(1.60)	25.5(2.50)

( ) represents standard deviation (n=3).

in leaf and stem tissue but did not affect the soluble reduced-N concentration in the root tissue (Fig. 3). The phosphorus deficiency reduced the proportion of reduced soluble nitrogen in leaf from 60% to 50%, but increased that in roots from 10% to 20% during the experimental period (Table 1).

The effect of phosphous deficiency on the ureide-N concentrations in the leaf, stem and root tissue are illustrated in Fig. 4. The ureide-N concentrations in leaf and stem tissue were significantly higher in control plants than those in phosphorous deficient plants but the ureide-N concentration in root tissue was not affected by phosphorus deficiency.

The proportion of ureide nitrogen in leaf of control plants was higher than that in phosphorus deficient plants, whereas, roots of phosphorus deficient plants contained a higher proportion of ureide nitrogen than that of control plants (Table 2). The excess accumulation of amino acids in the leaf of nitrate dependant plants under phosphorus stress resulted from restriction in utilization of soluble reduced-N (protein synthesis) in the leaves.[15-17] But in this experiment with nitrogen fixing soybean plant the higher(34%) ureide-N concentration in the leaf of the control plant was not due to lower ureide degradation and utilization efficiency in the leaf, because the amount of ureide flux from xylem to leaf was reduced by more than 80% in phosphorus deficient nitrogen fixing soybean plant under the similar growing conditions as this experiment.[13]

Enhanced retention of ureide in the root system (Fig. 4, Table 2) was largely responsible for the decrease in the ureide utilization efficiency in the leaf of phosphorus deficient plant, and indicated that transport of ureide-N out of the root symplasm into the shoot is restricted by phosphorus deficiency. Moreover, the restricted translocation from the root to shoot would severely limit the delivery of ureide to leave and thus leaf ureide degradation and *de novo* protein synthesis may be impaired by phosphorus deficiency. A majority of soluble reduced-N is associated with nitrogen pool cycling between the root and shoot.[18,19] Thus, the inhibition of translocation in phosphorus deficient plants apparently extended throughout the long distance transport system and encompassed different molecules originating from different endogeneous sources.

The general inhibition of endogeneous N translocation in phosphorus deficient plant may be related to decreased water flow through the root and the xylem. Radin and Eidenbock[20] reported the hydraulic conductance of plants decreased within several days after plants are supplied with suboptimal phosphorus. Decreased conductance evidently results from changes in

membrane properties in the root cortex.[21] Another possibility is that translocation of fixed nitrogen out of root is influenced by decreassed energy availability by phosphorus deficiency. Rufty *et al.*[5] reported that phophorus deficiency significantly reduced ATP content and energy charge of soybean root. Nonetheless a significant amount of evidence has established the energy dependence of transport process,[22-24] less is known about regulation of nitrogen translocation.

Acknowledgements

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인산결핍 조건하에서 질소고정식물체내의 Ureide 분배

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초록 : 인산 결핍이 질소고정식물체의 Ureide 농도 및 분배에 미치는 영향을 살펴보고자 *Bradyrhizobium japonicum* MN 110을 접종한 대두 식물체를 1.0 mM-P(대조구) 및 0.05 mM-P(결핍구)를 함유한 무질소 영양액을 처리, 재배하여 이식 28, 35, 42, 49일 후 수확하였다. 3주의 실험기간중 건물량은 대조구의 경우 8.9배, 결핍구의 경우 2.7배 증가하였다. 인산 결핍구에서 식물체의 전질소 및 인산의 함량은 이식 후 28일에 각각 80%, 40%, 49일에 각각 93%, 84% 감소하였으며 뿌리 및 근류보다 잎과 줄기의 질소 농도가 크게 감소하였다. 인산 결핍은 잎과 줄기의 수용태 및 ureide태 질소 농도를 감소시켰으나 뿌리의 경우 영향을 받지 않았으며 인산결핍구의 경우 전체 수용태 및 ureide태 질소의 함량중 뿌리 함유량 비율이 대조구에 비하여 증가하였다. 이러한 결과는 인산 결핍이 근류의 질소고정뿐만 아니라 고정질소의 상향 이동도 저해함을 나타낸다.

찾는말 : 질소고정, 인산결핍, ureide