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EFFECT OF PHOSPHORUS NUTRITION ON THE NODULATION, NITROGEN FIXATION AND NUTRIENT - USE EFFICIENCY OF *BRADYRHIZOBIUM JAPONICUM* – SOYBEAN (*GLYCINE MAX* L. MERR.) SYMBIOSIS

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Summary. Characterization of nodule growth and functioning, phosphorus status of plant tissues and host- plant growth of nodulated soybean (*Glycine max* L. Merr.) plants grown under different phosphorus conditions was studied in order to evaluate the role of phosphorus in symbiotic nitrogen fixation. Phosphorus deficiency treatment decreased the whole plant fresh and dry mass, nodule weight, number and functioning. Under conditions of phosphorus oversupply the decrease in plant growth, nodulation and acetylene reduction was stronger. Phosphorus deficiency significantly affected all phosphorus metabolites. Contents of different phosphorus fractions were decreased under the conditions of phosphorus deficiency.

Key words: nitrogen fixation, phosphorus partitioning, soybean

Abbreviations: AR – acetylene reduction, P_{tot} – total phosphorus, P_i – inorganic phosphorus, P_{org} – organic phosphorus, P_{lp} – lipid phosphorus, ~P – high energy phosphorus, P_{suc} – sugar phosphorus, P_{nucl} – nucleotide phosphorus

Introduction

Phosphorus (P) is essential macronutrient for plant growth and function. The requirements of host plants for optimal growth and symbiotic dinitrogen fixation processes for P have been assessed by determination of nodule development and functioning (Sa and Israel, 1991). The influence of P on symbiotic nitrogen fixation in leguminous

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plants has received considerable attention, but its role in the process remains still unclear. Robson et. al (1981) concluded that P nutrition increased symbiotic dinitrogen fixation in subterranean clover (*Trifolium subterraneum* L.) by stimulating host plant growth rather than by exerting specific effects on rhizobial growth or on nodule formation and function. The increase of whole plant growth and plant nitrogen concentration in response to increased soil P supply have been noted for several leguminous species including soybean (Andrew and Robins, 1969; Israel, 1987; Israel, 1993). Decreased specific- nitrogenase activity in nodules of P- deficient soybean plants was associated with decreased energy status of host plant cells of nodules. These latter observations imply specific involvement of phosphorus in symbiotic nitrogen fixation. However, the conversion of inorganic P into different forms of organic P is not known, especially concerning the formation and functioning of symbiotic nodules. Therefore, in the present study we tried to evaluate the effect of deficiency of P on the growth, nodulation and contents of different P metabolite fractions in the leaves and nodules of symbiotically grown soybean plants.

Materials and methods

Soybean plants were grown as a water culture in a naturally lit and heated greenhouse for 3 weeks. The composition of the nutrient solution was as described by Yamamoto and Yamagishi (1994), except for the potassium dihydrogen phosphate that was used as a source of P which was varied to produce different concentrations in the nutrient solution, namely: 0.1, 1.0 and 3.0 mM phosphate. Seeds of soybean cv. Beason were sterilised with 2% NaOCl and were germinated on moist paper for 72 h in Petri dishes. Roots of 3 day old seedlings were inoculated with *Bradyrhizobiun japonicum* strain 639 (NBIMCC) 10^8 viable cells/ml/cultivated on yeast manitol medium before transplanting the plants to pots with nutrient solution). A set of plants was used for assessment of nitrogenase activity of nodules formed after 27 days of growth by acetylene reduction (AR) assay (Hardy et al, 1968). For determination of P fractions only fresh tissue samples were used which were homogenized with 5 ml 10% $HClO_4$ at 4ºC. After centrifugation at 4000 rpm at 4ºC, the supernatant was collected for analysis of P fractions. The pellet was resuspended in 15 ml 5% $HClO_4$ and again centrifuged. The received solution was called the initial solution A. This solution was used for determination of total P, inorganic P, high energy P, P from sugars and P from nucleotides. By using activated charcoal, organic P compounds were separated from inorganic. For determination of total P, the aliquots from the initial solution A were ashed at 180ºC with 0.4 ml conc. $HClO_4$ and the ash was dissolved and appropriately diluted with distilled water. 10 ml initial solution A was incubated with 150 mg activated charcoal for 30 min at 4ºC. Nucleotides from the solution were absorbed on the charcoal and the solution was used for determination of Pi and total P after absorption of nucleot-

ides. The content of sugar P was calculated by subtraction from the total P/received after the charcoal absorption/ the content of inorganic P found in the solution A. The content of nucleotide P was calculated by subtraction from the total P the content of P received after the charcoal absorption.

For assay of the high energey P (~P), 400 mg charcoal was incubated with 25 ml initial solution A for 30 min at 4°C and the received suspension was centrifuged at 4000 rpm for 10 min. The charcoal pellet containing the macroergic ~ P was washed three times with distilled water to remove the residual inorganic Pi. ~P was extracted from charcoal with 5 ml 1 N HCl for 10 min in boiling water. All received fractions of P metabolites was converted into the inorganic form and analyzed by the colorimetric method of Lowry, Lopez and Skulatchov (1962). Appropriate aliquots were mixed with 5 ml 0.1 M acetate buffer pH 4.0, 0.5 ml 1% ammonium molybdate in 0.05 N H_2SO_4, 0.5 ml 1% Na-ascorbate. To avoid the delayin the conversion of the blue colour of molybdate- phosphoric complex, 1 mM $CuSO_4.5H_2O$ was added into the ascorbate solution. The blue colour of the complex was obtained after 10 min and the absorption was determined using spectrophotometer "Specol 11" at 620 nm.

Results and Discussion

Alteration of P supply in nutrient solution caused significant changes in phosphorus metabolism in both the host and the nodules of soybean The changes in P supply decreased nodule fresh and dry weight by almost 50%, in both P deficient and P oversupplied plants. The excess of P decreased nodule number up to the 35% in comparison with control. Nodulation of P-deficient soybean was less affected, but most of the nodules showed smaller size in comparison with the control. The whole plant total nitrogenase activitiy determined as AR rate were 55% and 47% of control for the P-deficient and, respectively, for oversupplied P plants (Table. 1).

Table 1. Effect of different P concentration on plant fresh and dry matter; nodulation rate and N_2 fixation

Treatment	Plant		nodule			
	Fwt g/plant	Dwt g/plant	Fwt g/plant	Dwt g/plant	Number plant	Total AR µM C_2H_4/gFwt/h
control						
1.0 mM P	10.55±1.38	1.37±0.18	0.85±0.14	0.13±0.02	37.25±15.3	17.1
stress						
0.1 mM P	9.49±0.96	1.28±0.23	0.64±0.09	0.10±0.02	32.38±1.83	9.3
3.0 mM P	8.65±1.48	1.03±0.20	0.54±0.13	0.08±0.02	24.13±0.18	6.2

data are means±SD

These observations indicated that both photosynthetic and dinitrogen fixation processes, were inhibited. Several physiological and metabolic features were associated with the lowering the N_2 fixing activity in the nodules under non-optimal P nutrient supply (Table. 1). First of all, this is the decrease of nodule mass and number. Secondly, the total nitrogenase activity measured as AR rate was also low under non-optimal P nutrition. And, finally, the deteriorated phosphate metabolism lead to impairment of photosynthetic and nitrogen fixing metabolism.

The analysis of phosphate fractions of total P_{tot}, inorganic P_i, organic P_{org}, lipid P_{lp}, high-energetic ~P, sugar P_{suc} and nucleotide P_{nucl} showed lower concentrations of all investigated metabolites in P-deficient nodules, with the exception of P_{nucl}, whose amount was higher in the P deficient leaves (Table 2). Data show that under P deficiency the most inhibited was leaf P_i (32% of control). But the content of total and organic P were also strongly affected. This may be a result of inhibition of the conversion of sugar P and lipid P in the deficient leaves. Surprisingly, the content of nucleotide P_{nucl} in the deficient leaves was increased (303% of the control nucleotide P quantity). The contents of all fractions studied were more negatively affected in the nodules than in leaves under P deficiency with the exception of inorganic and high energy P fractions. The less affected nucleotide and high-energy P in the P deficient nodules may be due to the fact that much more reserves of stored carbohydrates exist in bacteroids and infected cells under P deficiency (Sa and Israel, 1991). The possibility that ATP synthesis was limited by deprivation in Pi uptake can also be considered (Sa and Israel, 1991). P deficiency might have caused a negative effect on the processes of nitrogen fixation by decreasing nodule capacity to fix atmospheric N_2 as result of lowered nodule size.

As the changes in all the P fractions were more significant in the nodules than in leaves, we concluded that the effect of P deficiency on the nodule formation and functioning may be due to the limited exchange of assimilates between the shoot and nod-

Table 2. Content of phosphorus metabolites extracted from soybean leaves and nodules affected by different P nutrition

Treatment	Total P_{tot}	Organic P_{org}	Inorganic P_i	High energetic ~P	Sugar P_{suc}	Nucleotide P_{nuc}	Lipid P_{ld}
	µgP/gFwt	µgP/gFwt	µgP/gFwt	µgP/gFwt	µgP/gFwt	µgP/gFwt	µgP/gFwt
+P leaves	2531.3	2377.0	153.1	3.3	996.0	229.8	1137.0
+P nodules	3656.3	3595.6	60.7	2.7	1331.1	869.9	1391.9
-P leaves	1689.5	1639.6	49.4	1.9	446.3	694.1	497.2
-P nodules	1968.8	1930.1	39.1	2.6	550.7	786.5	589.9

* Control treatment 1 mMP, +P , Stress treatment 0.1 mM, - P;

** Data are means of 3 replicates, standard error /not shown/ was 5.6%.

ules which is favoured by the inhibition of leaf photosynthesis through the decrease in the free available inorganic P. The P treatments did not significantly alter the of ~P concentrations in the leaves and nodules but increased the other nucleotide P. These results indicate that nucleotide metabolism under P deficiency may be the main target of deteriorated P nutrition in N_2 fixing plants which fact deserves more attention in future experiments.

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