INOCULATION BY *BRADYRHIZOBIUM JAPONICUM* STRAINS WITH DIFFERENT P-SOLUBILIZING ACTIVITY AND ITS EFFECT ON SOYBEAN YIELDS

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The ability to release phosphorus from hardly soluble compounds was determined in three *Bradyrhizobium japonicum* strains D576, D574 and D216. These strains were then tested for association with soybean roots (*Glycine max* L.) in a pot experiment with soil. The pots were fertilized by non soluble Gafsa rock phosphate in a quantity corresponding to 90 kg per hectare. The effective phosphorus solubilizing strain D574 increased the shoot dry weight in soybean, as well as the quantity of labile phosphorus in soil.

Bradyrhizobium; *Rhizobium;* nodule bacteria preparation; soybean; solubilization of phosphorus; rhizosphere microflora

Only a small part of phosphorus added into the soil in mineral fertilizer form is used up by plants. The effect of P-fertilizing is estimated from 10 to 30 %. In fact, 70 to 90 % of added phosphorus get transformed into hardly soluble forms which are difficult to take by plants. As phosphorus is relatively little used up, regular and high doses of mineral fertilizers are used to facilitate high yields.

One of the possibilities how to increase the effectiveness of mineral P--fertilizing and to improve utilization of soil phosphorus is to use the activities of soil microflora.

The ability to release phosphorus from hardly soluble compounds was found also in bacteria of the Bradyrhizobium (Reichlová, 1972) and Rhizobium geni (Badr El-Din et al., 1985; Dhingra et al., 1987).

The effect of three *Bradyrhizobium japonicum* strains with different P-solubilizing activity on soybean yields was observed as well.

MATERIAL AND METHODS

Three *Bradyrhizobium japonicum* strains D576, D574 and D216 from the Czechoslovak Rhizobia collection were used for inoculation. These strains

were first tested for their P-solubilizing activity in submersion shaked cultures.

Inoculum was prepared from a collection strain destined for testing in liquid culture. 1 ml of prepared inoculum was pipetted into 100 ml of sterile nutrient solution (D o m e y , 1987), 50 mg of non soluble $Ca_3(PO_4)_2$ was added. Cultivation went on in flasks for 7 days at 28 °C by constant shaking. Each variant had three replications. Microscopic control of the medium was done at the beginning and at the end of incubation. The content of soluble phosphorus was determined according to M u r p h y , R i l e y (1962) and M a c h á č e k , K o t e k (1978).

Soybean (*Glycine max* L.), variety Sluna, was used as experimental plant. One sprouted soybean seed was planted into a plastic pot filled with 400 g of sieved brown soil from a plot of the Research Institute for Crop Production Prague-Ruzyně. Each pot was fertilized by non soluble Gafsa phosphate in a quantity corresponding to 90 kg of phosphorus per hectare. Each variant was grown in 14 replications. After sowing the seeds were inoculated by 20 ml (1.10^9 cells/ml) of suspension of the respective *Bradyrhizobium japonicum* strain. Control without inoculation was not introduced into the experiment as the Ruzyně soil does not contain native populations of *Bradyrhizobium japonicum* and N-uptake into the plant would be in this case a limiting factor.

The pots were placed in a glasshouse at day temperature of 25 to 30 °C, night temperature of 15 to 18 °C and photoperiod of 18 hours. The experiment was completed in the stage of full flowering and the following factors were analysed: shoot dry weight, root dry weight, number of nodules and quantity of labile phosphorus in soil (B r a y , K u r t z , 1945; M c L e a n , 1985). Statistic evaluation was done by a one way variance analysis and consecutive comparison of mean levels by Tukey's method.

RESULTS AND DISCUSSION

P-solubilizing activity of three *Bradyrhizobium japonicum* strains D576, D574 and D216 was analysed in the first part of the experiment. The content of solubilized phosphorus in a solution of submersion shaked cultures of inoculated suspensions of individual strains (Tab. I) was determined.

The results make it evident that D576 and D216 strains did not release any phosphorus. These strains are lacking the P-solubilizing activity. On the contrary, the D547 strain shows a large P-solubilizing activity.

From the point of view of nitrogenase activity, comparatively effective strains were selected.

I. Solubilized phosphorus content in submersion shaked cultures

Bradyrhizobium japonicum strains	P (mg/l)
D576	0
D574	45.7
D216	0

II. Yield of shoot and root dry weight, number of nodules and quantity of labile phosphorus in soil in soybean inoculated by different strains

Brad. japon. strain	Shoot dry weight (g/plant)	Roots dry weight (g/plant)	Number of nodules per plant	Quantity of labile P (mg/kg)
D576	0.51 A*	0.08 NS**	12.1 NS	48.1 AB
D574	0.60 B	0.09	13.1	56.1 B
D216	0.54 AB	0.09	11.9	42.5 A

* levels in the column designed by the same letter do not differ in a statistically significant way ($P = 0.05$)

** statistically not significant differences

In the second part of the experiment, the strains were tested in association with soybean roots (Tab. II).

The present research was based on the fact that both factors, accessible phosphorus and inoculation, increase nodulation and nitrogenase activity and may thus influence the soybean yields as well. As strains with comparable nitrogenase activity were chosen for inoculation, the dry weight increase may be at least partly due to their different P-solubilizing activity. As the phosphorus was applied in the form of hardly soluble Gafsa rock phosphate, it may be supposed that the active phosphorus solubilizing strain D547 made the phosphorus partly available. This is proved also by a higher level of labile phosphorus in soil in this variant.

The results of our experiment confirm conclusions of a number of authors mentioning in their papers the ability of *Rhizobium* and *Bradyrhizobium* bacteria to release phosphorus from hardly soluble compounds (B a d r E l - D i n et al., 1986; D h i n g r a et al., 1988; D o w n e y , V a n K e s s e l et al., 1990; K a b e s h et al., 1990).

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MIKANOVÁ, O. - KUBÁT, J. (Výzkumný ústav rostlinné výroby, Praha-Ruzyně):

Inokulace kmeny *Bradyrhizobium japonicum* s různou P-solubilizační aktivitou a jejich vliv na výnosy sóje.

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Jednou z možností jak zvýšit účinnost hnojení fosforečnými hnojivy a zlepšit čerpání fosforu z půdy je využití aktivity půdní mikroflóry (Domey, 1987).

Schopnost uvolňovat fosfor z těžko rozpustných sloučenin byla zjištěna také u bakterií rodu Bradyrhizobium (Reichlová, 1972) a Rhizobium (Badr El-Din et al., 1985; Dhingra et al., 1987).

V práci byl sledován vliv tří kmenů *Bradirhizobium japonicum* s různou P-solubilizační aktivitou na úrodu sóje.

K inokulaci bylo použito tří kmenů *Bradyrhizobium japonicum* z Čs. sbírky Rhizobií D576, D574, D216. Tyto kmeny byly nejprve testovány z hlediska P-solubilizační aktivity v submersních třepaných kulturách. Obsah rozpustného fosforu byl stanoven podle autorů Murphy, Riley (1962) a Macháček, Kotek (1978) (tab. I).

Tyto kmeny byly dále testovány v asociaci s kořeny sóje (*Glycine max* L.) odrůdy Sluna v nádobovém pokusu se zeminou. Květináčky byly hnojeny nerozpustným fosfátem Gafsa v množství, které odpovídalo 90 kg P na hektar. Nádoby byly umístěny ve skleníku. Pokus byl ukončen ve fázi plného květu a byly vyhodnoceny: sušina NH, sušina kořenů, počet hlízek a množství labilního fosforu v půdě (Bray, Kurtz, 1945; McLean, 1984). Statistické hodnocení bylo provedeno jednocestnou analýzou variance a následným srovnáním mezi průměry pomocí Tukeyovy metody (tab. II).

Z výsledků vyplývá, že kmeny D576 a D216 neuvolnily žádný fosfor. Jedná se tedy o kmeny bez P-solubilizační aktivity. Naproti tomu kmen D574 vykazuje značnou fosfor solubilizující aktivitu. Z hlediska nitrogenázové aktivity byly vybrány kmeny srovnatelně efektivní.

Nynější výzkum byl založen na skutečnosti, že oba faktory, přístupný fosfor i inokulace, zvyšují nodulaci a nitrogenázovou aktivitu a mohou tedy ovlivňovat i výnosy u sóje. Vzhledem k tomu, že k inokulaci byly vybrány kmeny vykazující srovnatelnou nitrogenázovou aktivitu, lze zvýšení sušiny alespoň částečně přičítat jejich rozdílné P-solubilizační aktivitě. Protože fosfor byl aplikován ve formě těžko rozpustného Gafsa-fosfátu, existuje předpoklad, že aktivní fosfor solubilizující kmen D574 částečně fosfor zpřístupnil. Tomu odpovídá i zvýšené množství labilního fosforu v půdě u této varianty.

Výsledky našeho pokusu potvrzují zjištění řady autorů, v jejichž pracích je schopnost bakterií rodu Rhizobium a Bradyrhizobium uvolňovat fosfor z těžko rozpustných sloučenin také uvedena (Badr El-Din et al., 1986; Dhingra et al., 1988; Duwney, Van Kessel, 1990; Kabesh et al., 1990).

Bradyrhizobium; *Rhizobium;* očkovací preparát; sója; solubilizace fosforu; rhizosferní mikroflóra

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