INFLUENCE OF PHOSPHORUS FERTILIZATION AND *BRADYRHIZOBIUM JAPONICUM* INOCULATION OF SOYBEAN ON ACTIVITIES OF ACID PHOSPHATASES IN ROOTS AND RHIZOSPHERE SOIL UNDER PHOSPHORUS AND WATER LIMITED CONDITIONS

INFLUENȚA FERTILIZĂRII CU FOSFOR ŞI INOCULĂRII CU *BRADYRHIZOBIUM JAPONICUM* ASUPRA ACTIVITĂȚII FOSFATAZEI ACIDE ÎN RĂDĂCINI ŞI SOLUL RIZOSFERIC LA SOIA ÎN CONDIȚII LIMITATE DE FOSFOR ŞI UMIDITATE

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Abstract. *A pot experiment was conducted to examine the impact of Bradyrhizobium japonicum rhizobacteria along with P fertilizer on the activity of acid phosphatases (APase) in roots and rhizosphere soil of soybean under phosphorus and water limited conditions. Non-inoculated and inoculated soybean plants (cv Horboveanca) were supplied with three levels of P: 0 mg P/kg soil, (P0, insufficiency P), 20 mg P/kg (P20, medium) and 100 mg P/kg (P100, sufficient). At the flowering stage a set of plants was subjected to moderate drought stress for 12 days. Experimental results have shown that the root enzyme activity was much higher in the treatment without fertilization compared to those fertilized with phosphorus irrespective of soil moisture level. Inoculated plants with B. japonicum exhibited greater acid phosphatases activity in roots than non-inoculated plants. The same trend was observed in soil acid phosphatase activity under well-watered and water deficit conditions.*
Key words: acid phosphatase, *B. japonicum*, moisture, phosphorus, soybean

Rezumat. *S-a organizat un studiu în vase de vegetație pentru a evalua influența bacteriilor Bradyrhizobium japonicum aplicate separat sau în combinare cu fosforul asupra activității fosfatazei acide în rădăcini şi solul rizosferic la plantele de soia, cultivate în condiții limitate de fosfor şi umiditate a solului. Plantele (cv Horboveanca) neinoculate şi celea inoculate le-au fost administrate diferite doze de fosfor: 0 mg P/kg sol, (P0, nefertilizat), 20 mg P/kg (P20, mediu) şi 100 mg P/kg (P100, suficient aprovizionat). Plantele au fost supuse deficitului de apă la faza de înflorire pentru 12 zile. Rezultatele experimentale au demonstrat că activitatea enzimei a fost mai înaltă în variantele fără fertilizarea chimică comparativ cu cea depistată la fertilizare indiferent de regimul de umiditate a solului. Aplicarea B. japonicum a majorat activitatea enzimei în rădăcini față de plantele neinoculate. Aceiaşi tendință s-a observat şi la nivel de activitate în solul rizosferic.*
Cuvinte cheie: *B. japonicum*, fosfataza acidă, fosfor, umiditate, soia

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INTRODUCTION

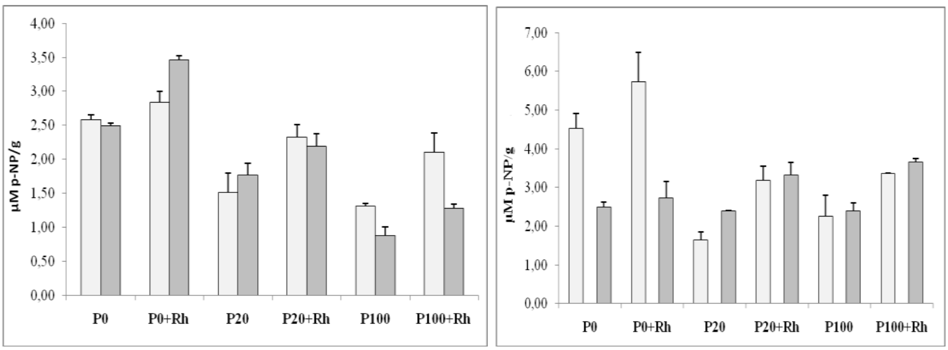
Nowadays the farmers are largely cash limited and mineral fertilizers being costly, restricts their capacity to purchase fertilizers and hence it is needed to develop sustainable agricultural production especially under scarce water and nutrient environments. The low yields of legumes are partly due to infertility caused by carbonated soils which have low nutrient contents, particularly of available phosphates (Andries, 2007), insufficient water supply and compatible *Rhizobium* for adequate N2 fixation. The microorganisms application as biofertilizers is considered a promising alternative that supports an effective approach for improving plant nutrition, decreasing agricultural costs, maximizing crop yield by providing them with available nutrients, particularly P (Lugtenberg and Kamilova, 2009). Almost half of the microorganisms present in soil or on plant roots possess the ability to mineralize organic phosphorus through the action of phosphatases (Tarafdar and Classen, 1988). These enzymes catalyze the cleavage of mineral P from organic phosphate esters, in acidic and alkaline soils that are poor in P (Nannipieri *et al*, 2011), thus making P more available in these soils. Rhizobium inoculation appears to increase P use efficiency in field grown soybean plants and in faba bean under controlled conditions (Boudanga *et al*, 2015). The aim of this study was to asses root acid phosphatase, rhizosphere acid and alkaline phosphatase activities of soybean in relation to application of *B. japonicum* and P fertilizer under water limited conditions.

MATERIAL AND METHOD

To accomplish the study's objectives a pot experiment was conducted under controlled soil moisture conditions. The soil was represented by chernoziom carbonated with low available phosphates. The experiment was laid out in a randomized complete block design, with four replicates for each treatment. Phosphorus (P) was administered to soil before the sowing. Soybean seeds (*cv Horboveanca)* were inoculated with suspension of rhizobacteria *Bradyrhizobium japonicum* (Rh) before sowing. The seeds of soybeans were grown in experimental pots (10L capacity) filled with non-sterilized soil: sand mixture (3:1). Water status was monitored by weighing the pots and water was supplied to maintain soil moisture at 70% of water holding capacity (WHC) before exposing the plants to water stress. The soil moisture content was reduced through natural evapotranspiration and then the water stress was maintained at 35% of WHC for 12 days. Plant morphological and physiological parameters were determined at the end of drought. The acid and alkaline phosphatase activity in soil was performed as described by Tabatabai and Bremner (1969). The root and nodules acid phosphatase activity was analyzed by procedure described by Kaous S. *et al*, (Kaous *et al*, 2009). Soil pH in distilled water (1:2.5 v/v soil:water) was determined in rhizosphere soil. Subsamples of soil were air-dried and used for measuring soil available P. The content of available soil phosphates was determined using the Murphy and Riley's method (Murphy and Riley, 1962). Statistical analyses were carried out using a STATISTICA 7 software program.

RESULTS AND DISCUSSIONS

The use of microorganisms provides better plant nutrition and promotes plant growth. It is well documented that leguminous species, particularly soybean (*Glycine max* L) have a higher demand in phosphorus nutrition. Nitrogen-fixing microorganisms besides their ability to assimilate nitrogen from atmosphere have a contribution for promoting the growth and nutrition of crops.



A B

Fig. 1 Effect of P fertilizer and *B. japonicum* inoculation on the root (A) and nodules (B) acid phosphatase activity of soybean under well water (grey bar) and water stress (dark bar). Bars represent the means with SE.

Experimental results showed that treatment with such kind of rhizobacteria affected the activity of acid phosphatases in roots and rhizosphere soil. It was observed that under water deficit conditions the phosphatase activity was enhanced in roots of inoculated plants grown under P deficiency in comparison to uninoculated plants (fig. 1A). The same trend was revealed in treatments with combined application of rhizobacteria and P in well-watered and drought stressed plants. Hence, it was found that soybean with application of *B. japonicum* had higher root phosphatase activity than their uninoculated counterparts. As a consequence, more soil available phosphorus could be released with an increase in rhizobacteria mediated acid phosphatase resulting in partial alleviation of drought stress as well as P deficiency (Stancheva *et al,* 2008). According to investigations of Araujo A. (2008) bean plants subjected to P-deficiency increased the activities of phosphatases and phytases in nodules. This response constitutes an adaptative mechanism for N2-fixing legumes to tolerate P deficiency by improving the utilization of the P within nodules. Similarly, in our study the utilization of rhizobacteria had beneficial impact on the activity of acid phosphatase of nodules in relation to water supply (fig. 1B). The P insufficiency treatment (P0) caused an increase in nodule phosphatase activity under inoculation when compared to the P sufficient supply. In addition, it was established that the enzyme activity increased in treatment with *Bradyrhizobium japonicum* application together with phosphorus fertilizer.

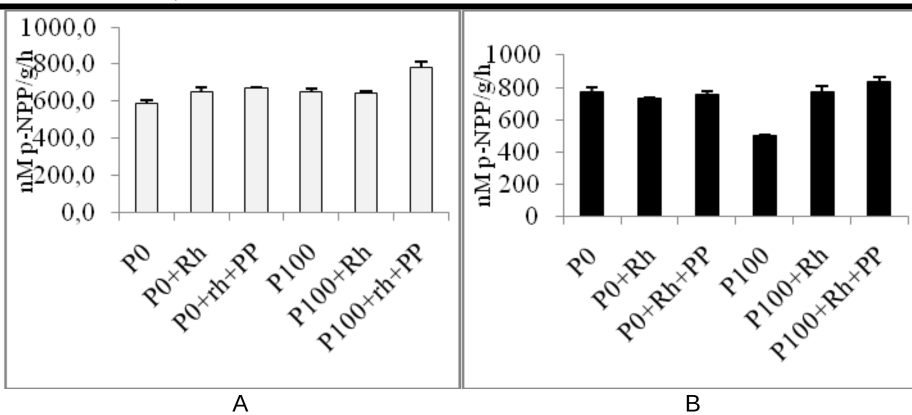
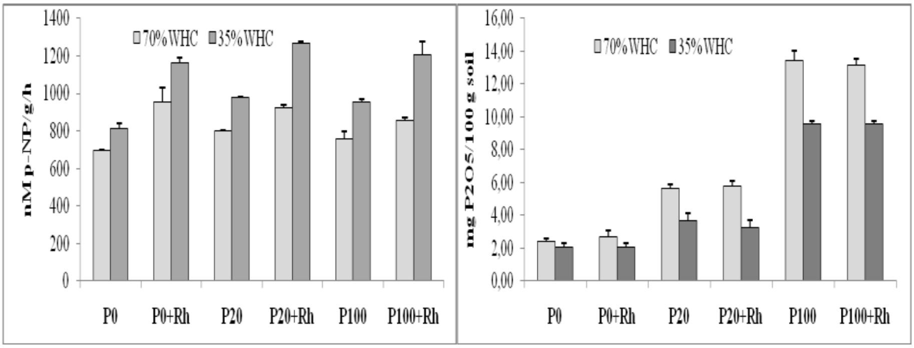


Fig. 2 Effect of P fertilizer and *B. japonicum* inoculation on the soil acid phosphatase activity under normal water (A) and water deficit (B). Bars represent the means with SE.

The P fertilization alone decreased this parameter irrespective of soil moisture level compared to treatment without fertilization. We agree with the assumption that the increase of the acid phosphatases activities in nodules and roots may constitute a biological option to improve P assimilation and to attenuate partial P deficiency (Boudanga *et al*, 2015).

Soil enzyme activities in the rhizosphere soils of legumes serve many fundamental biochemical roles. The experimental data showed significant differences in acid and alkaline phosphatases activities in soil with the application of *B. japonicum* bacteria and P (Fig. 2 and 3). In general, increases in acid and alkaline phosphatase activities of soil were recorded with the inoculation of *Bradyrhizobium japonicum.* This may be attributed to increased plant roots growth (Lugtenberg and Kamilova, 2009), which in turn stimulated the proliferation of soil microorganisms in the rhizosphere. Their influence was observed in both soil water regimes well-watered and water deficit. Likewise, the impact of rhizobacteria was evident in the pots with P fertilization, but the increases were lower compared to the unfertilized treatment. We suppose that the roots of *Glycine max* L. supplied with P in doses 20 and 100 mg per kg of soil secreted less phosphatases compared to the treatment without fertilization. In general, drought contributed to the increase of the enzyme activity of soil. Probably, water deficit diminished the content of available phosphates in the soil. The application of phosphorus in low dose (20 mg P/kg soil) alone or in combination with *B. japonicum* stimulated the alkaline phosphatase activity of rhizosphere soil in comparison to unfertilized and non-inoculated treatments (fig. 3B). The use of this kind of rhizobacteria increased the alkaline phosphatase activity in soil by 28% in pots of normal soil water regime and by 38% in the rhizosphere soil of plants subjected to water deficit. Under utilization of rhizobacteria together with P fertilizer the increases were less and was 11.8% under optimal water regime and 24.4% under moderate drought conditions. The administration of a higher dose of P (100 mg/kg) did not change this parameter comparing with a low dose.



A B

Fig. 3 The soil alkaline phosphatase activity (A) and the phosphates content (B) in the rhizosphere soil of soybean inoculated with *B. japonicum* and P fertilization grown under sufficient moisture (grey bar) *versus* deficit moisture (black bar). Data are means ± SE.

In agricultural soils the solubilization of inorganic phosphate is closely associated with the activity of soil microorganisms including rhizobia (Tarafdar and Claassen, 1988). The content of available phosphates in the rhizosphere soil was significantly higher in P fertilized treatments than in the treatment without fertilization (fig. 3B). The use of *B. japonicum* had no significant impact on the content of available phosphates in soil. Only a moderate increase (by 10%) of mobile phosphates in rhizosphere soil was observed in treatment with rhizobacteria administration under insufficient P supply.

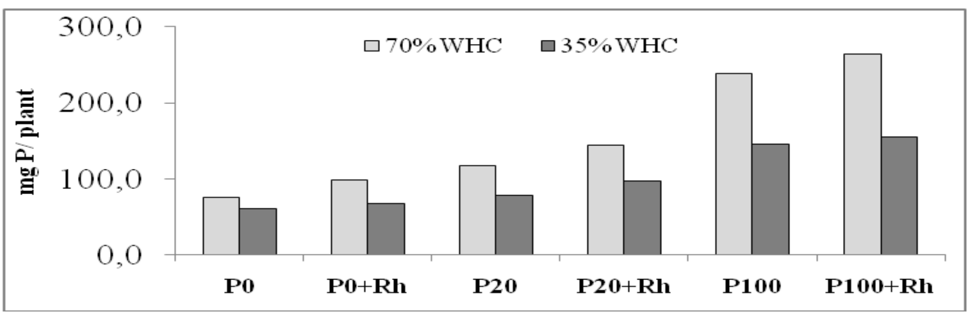


Fig. 4 The influence of rhizobacteria application and P fertilization on phosphorus uptake of soybean in relation to soil moisture conditions.

This increase was associated with a small decrease of pH in the rhizosphere soil. In this study, the values of soil pH were registered in relation to rhizobacteria application and P fertilization (data are not shown). There was only a trend of decreasing soil pH as the result of the application of rhizobacteria under limited water condition. An insignificant increase of this parameter was observed in treatment with rhizobacteria in combination with a low dose of P. The estimtion of P uptake revealed that the supplemental nutrition affected this parameter in both water soil regimes (Fig. 4). But its effectivness was more pronounced on plants that were not limited with water. The rhizobacteria application increased P acquisition by plants especialy grown in soil with P deficiency. Thus, the increase of phosphatases activities under application of rhizobacteria would have a beneficial influence on growth and tolerance of legumes to abiotic factors.

CONCLUSIONS

1. The inoculation of soybean plants with *Bradyrhizobium japonicum* grown on low phosphorus fertility increased root and soil acid phosphatases activity and P uptake of plants in comparison with the uninoculated treatment. The enzymes activities were lowest under P fertilization without rhizobacteria administration.

2. The application of *B. japonicum* in P-deficit soil alone or together with a low dose of phosphorus fertilizer improved the drought tolerance of plants through modulation of the root acid and alkaline phosphatases as well as soil rhizosphere, thus facilitating phosphorus acquisition.

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