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RESPONSES OF ACID PHOSPHATASE ACTIVITY ON THE ROOT SURFACE AND RHIZOSPHERIC SOIL OF SOYBEAN PLANTS TO PHOSPHORUS FERTILIZATION AND RHIZOBACTERIA APPLICATION UNDER LOW WATER SUPPLY

Vladimir ROTARU

Institute of Genetics, Physiology and Plant Protection, 20 Padurii Str. MD 2002, Chisinau, Republic of Moldova

Corresponding author email: rotaruvlad@yahoo.com

Abstract

Plant growth-promoting rhizobacteria (PGPR) are known to influence plant nutrition and growth by various direct or indirect mechanisms. Acid phosphatases (APase) produced by the roots of plants and microorganisms plays an important role in inorganic phosphate (Pi) acquisition. The present study was undertaken to evaluate the effects of phosphorus (P) and rhizobacteria suspension of Pseudomonas fluorecens and Azotobacter chroococcum application on root surface and soil rhizosphere acid phosphatase activities of two soybean (Glycine max. L. Merr.) cultivars. The pot experiment was conducted in a greenhouse under controlled soil moisture conditions and plants were cultivated on soil-sand mixture. Bacterial and phosphorus fertilization plants were subjected at flowering stage to temporally drought conditions (35% WHC) for 12 days. Both soybean cultivars Zodiac and Horboveanca supplied with rhizobacteria showed significantly ($p < 0.05$) higher root surface acid phosphatase activity than no treated plants irrespective of the soil water regime. But the activity of acid phosphatase in rhizothere soil under Zodiac was higher than under Horboveanca. Experimental results revealed a significant positive association between acid phosphatase activity in soil and roots with PGPR application, indicating the role of these enzymes in P nutrition of soybean. These results indicate that the application of rhizobacteria consortium pseudomonas fluorescens and azotobacter chroococcum plays an important role in modulation of root surface acid phosphatase activity of soybean and these could have a beneficial impact on P acquisition by possibly mobilizing organic P, but their beneficial effect must be tested under field conditions.

Key words*: Glycine max., acid phosphatase, phosphorus, rhizobacteria, drought.*

INTRODUCTION

Phosphate deficiency is considered to be one of the major environmental factors affecting plant growth, metabolism and productivity (Schachtman et al., 1998; Hammond et al., 2004; Li et al., 2008). It was well demonstrated that plants are able to utilize only a small portion of phosphoric fertilizers that are applied, as much of it is rapidly converted into insoluble complexes in soil (Rodríguez and Fraga, 1999; Cisse and Amar, 2000). Organic P comprises 30-80% of total P in most agricultural soils.

Among the mechanisms that contribute to the increase of Pi availability in soil are the exudations of organic acids or enzymes (like acid phosphatases) into the rhizosphere, as well as symbiosis with microorganisms (George et al., 2005; Tran et al., 2010; Duff et al., 1994; Tarafdar and Marschner, 1995; Gilbert et al., 1999).

Acid phosphatases (orthophosphoric monoester phosphohydrolyzes; EC 3.1.3.2, APases) belong to a broad group of enzymes that catalyze the hydrolysis of different phosphor-monoesters at low pH (Duff et al., 1994; Yadav and Tarafdar, 2001). The production of extracellular APases and secretion from roots rely on the environmental conditions, the physiological state of the plant, age or root type and architecture (Yadav and Tarafdar, 2001). It is well known that acid phosphatases, which catalyze the hydrolysis of organic phosphate compounds, are present in the rhizosphere of most plants (Shen et al., 2005; Tarafdar and Jungk, 1987). The use of PGPR has an important role in improving plant nutrition, particularly on soils of low fertility (Richardson et al., 2001). However, there is scarce information regarding the mechanisms of microorganisms that contribute to improve the availability of phosphates for plants.

Soybean has an important role in sustainable agriculture development and it is an essential source of vegetable protein and oils in the world. However, its production is largely limited by the phosphorus deficiency in many regions, in particular in the Republic of Moldova (Andries, 2011). The effects of PGPR on crops have been studied typically under normal water soil conditions. Actually these two major environmental constraints: phosphorus deficiency and low moisture of soil coexist in field conditions. Therefore, the objective of this study was to compare two soybean cultivars (differing in productivity and response to P fertilization) in terms of acid phosphatase activities on root surface and rhizosphere soil under fertilization with P or PGPR in relation to soil moisture regime. The identification of differences among acid phosphatase activities might be useful for selection of soybean varieties more tolerant to unfavorable environmental conditions.

MATERIALS AND METHODS

A pot experiment was conducted under controlled soil moisture regime. Two soybean (*Glycine max* L. Merr) cultivars were used in this investigation, namely Zodiac and Horboveanca differing in potential productivity. The soil was carbonated chernoziom with pH 8 and low available phosphate (1.8 mg/100g soil). The soil was sieved and then mixed with sand at the ratio 3:1. Ten kilograms of soil was put into each plastic pot. Macro- and micronutrients were thoroughly mixed with soil. The P levels (added as $Ca(H_2PO_4)_2$ were 0, 20 and 100 mg P/kg soil-sand mixture. The suspension of bacteria strains *Pseudomonas fluorecens and Azotobacter chroococcum* was applied by spraying the soil with bacteria suspension and then thoroughly homogenate.

Before the sowing the soybean seeds were treated with bacteria *bradyrhizobium japonicun*. The two water treatments were at 70% of water holding capacity (WHC) as control and at 35% WHC for 12 days as drought. The water deficit was initiated at the flowering stage. Soil moisture at the desired level was adjusted by watering the pot to the designated weight. Plants were harvested at the end of the drought period. The APase activity on root surface was analyzed using the modified method of Tang H. and co-workers (Tang et al., 2013). The soil adhering to roots was collected and then analyzed for acid phosphatase activity (Zhang et al., 2010). The enzyme activity was measured in μmol p-nitrophenol released from p-nitrophenylphosphate solution in 1 g soil within 1 h (μmol p-nitrophenol g^{-1} h^{-1}). Analysis of variance was performed with the GLM general Linear Model procedure of SPSS version 8.

RESULTS AND DISCUSSIONS

Root growth and its exudates are important for acquisition of P that is immobile in soil. Phosphatases released by plant roots or soil microorganisms can mineralize organic P (Tarafdar and Claassen, 1988), thus increasing P availability. Experimental results revealed a large variation in phosphatases activity upon application of bacteria suspension *pseudomonas fluorecens and azotobacter chroococcum* regardless of soil moisture levels. Likewise, the response of root surface APase activity of soybean to phosphoric fertilizer varied with genotypes as well as soil moisture levels. The root surface APase activity was significantly higher in Horboveanca than in Zodiac in all treatments under well watered conditions (Figure 1). The root surface APase activity of Zodiac after application of suspension bacteria *pseudomonas fluorecens and azotobacter chroococcum* was about 30% higher than that at unfertilized plants under normal water conditions (Figure 1). In Horboveanca, only APase activity on the root surface increased with P application under low water supply (Figure 2). Our experimental results are consistent with the study demonstrating that inoculation of *Aspergillus* strains improved P uptake by plants and availability of phosphates in soil (Tarafdar et al., 1996).

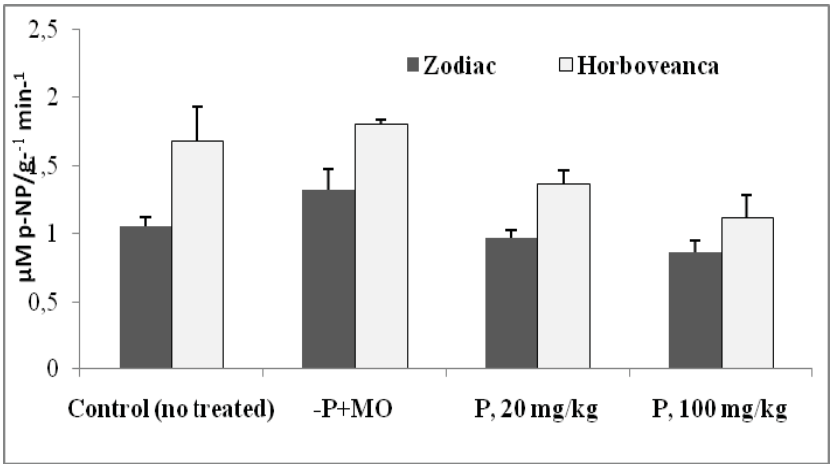

Figure 1. Effects of P fertilization and microorganisms (MO) application on the root surface acid phosphatases activity of two soybean cultivars grown under normal soil moisture conditions (70% WHC). Each value represents the mean of three replicates ± S.E.

Our data also showed that APase activity decreased under P fertilization only for Zodiac (Figure 2), which was consistent with the result of Gaume and researchers (Gaume et al., 2001). The use of PGPR increased significantly the APase activity on the root surface in P-efficient cultivar Zodiac, under optimal moisture regime. While in P-inefficient cultivar Horboveanca the APase activity increased but to a lesser extent (8.5% in Horboveanca, compared to 20.5% in Zodiac). This may be attributed to the increased growth of plant roots (Tarafdar et al., 1996), which in turn stimulated the proliferation of soil microorganisms in the rhizosphere.

Furthermore, the administration of biofertilizers had beneficial impact on root surface acid phosphatases activities of both cultivars subjected to water deficit conditions.

We can conclude that the biofertilizers administration had positive impact on root surface acid phosphatase activities of both cultivars. Under drought condition, the application of PGPR increased the root surface acid phosphatase activity by 13%. Therefore, drought reduced the beneficial influence of the rhizobacteria.

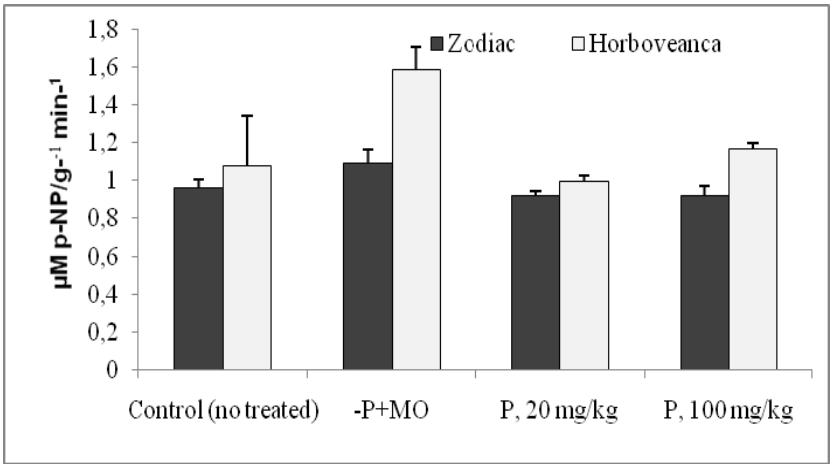

Figure 2. Effects of P fertilization and microorganisms (MO) application on the root surface acid phosphatases activity of two soybean cultivars grown under suboptimal water regime (35% WHC). Each value represents the mean of three replicates ± S.E.

Increased APase in roots of Zodiac may help hydrolyze the organic P pool in soil, and therefore enhance the availability of phosphates. Our results are consistent with others where a positive relationship has been reported between root APase activity and P uptake from organic P in bean (Helal, 1990) and barley (Asmar et al., 1995).

Mineral phosphorus fertilization and the application of PGPR showed also effects on phosphatase activities in the rhizosphere soil (Figures 3 and 4). The positive influence of microorganisms was observed on rhizosphere soil acid phosphatase activity and this parameter increased from 0.69 μM (no treated plants) to 1.08 μM/g/h for Zodiac under normal soil moisture regime.

The mineral fertilizer didn't have significant effect on rhizosphere soil acid phosphatase activity under Horboveanca when plants were subjected to drought. Very small changes in soil acid phosphatase activity were observed under sensitive soybean cultivar Horboveanca regardless of soil moisture regime. However, there was a pronounced effect after the rhizobacteria application in soil with Zodiac under normal water conditions. In such environment the rhizosphere acid phosphatase activity increased by 36% over the control treatment without fertilization. Probably, these changes in the rhizosphere could consequently affect plant nutrition. The study of Marschner et al. (2007) has shown that in low-phosphorus conditions, P uptake of wheat was in significant positive correlation with rhizosphere acid phosphatase activity. It is necessary to note, in our study, the lack of significant changes of pH in the rhizosphere soil was observed, which indicated that soybean cultivars Zodiac and Horboveanca do not respond to phosphate starvation *via* increase of protons or organic acids exudation from the roots, at least in our experimental conditions (data not shown). Tang et al (2007), also, demonstrated that soybean did not excrete protons in the rhizosphere.

Thus in the current study there were not significant changes of pH in water suspension of soil in relation to phosphorus and bacteria application. Likewise, the acidification of rhizosphere was not observed by Gaume et al. (Gaume et al., 2001) in corn plants. Hence, a significant increase of phosphatase activities in soil was observed after the PGPR.

Stronger impacts of PGPR in the unfertilized soil might be caused by the P deficiency in the soil since the phosphatase secretion is induced by low P supply (Helal and Dressler, 1989).

Compared to the untreated soils, the addition of phosphorus and biofertilizers improved P nutrition of both soybean cultivars regardless of soil moisture levels (data not shown). Therefore, increased secreted APases can contribute to enhancing the P uptake and utilization under P limited conditions.

The trend in soil enzyme activity was similar between two genotypes, but acid phosphatase activity in soil under P-efficient genotype Zodiac was significantly higher than that of P-inefficient Horboveanca genotype ($p<0.05$; Figure 4).

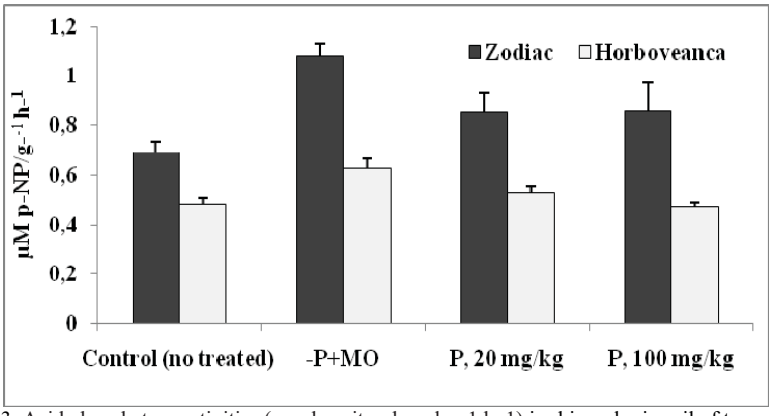

Figure 3. Acid phosphatase activities (μmol p-nitrophenol g–1 h–1) in rhizospheric soil of two soybean cultivars grown under P and rhizobacteria application in normal soil moisture conditions (70% WHC). Data are means ± S.E. of three replicates

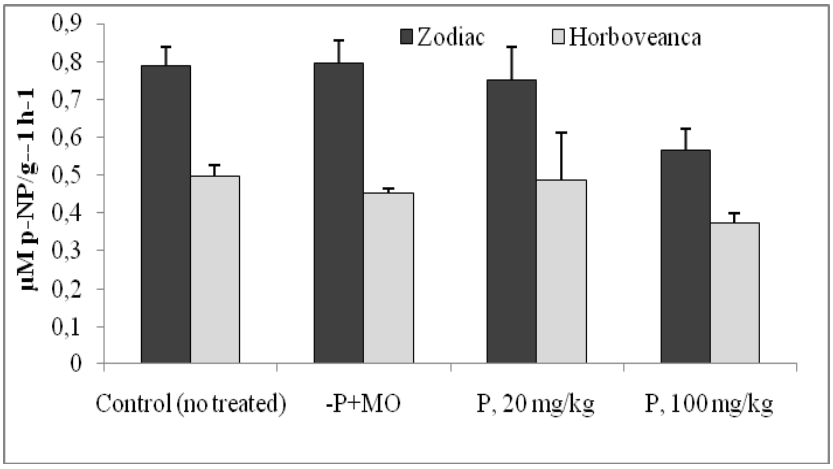

Figure 4. Acid phosphatase activities (µmol p-nitrophenol g–1 h–1) in rhizosphere soil after phosphorus fertilization and application of PGPR of two soybean cultivars under low water supply (35% WHC). Data are means ± S.E. of three replicates

CONCLUSIONS

The present results demonstrate that the application of the rhizobacteria consortium *pseudomonas fluorecens and azotobacter chroococcum* increased the acid phosphatase activity on surface roots and in the soil rhizosphere of soybean. However, the effect of PGPR was determined by soybean cultivar. The APase activity levels were significantly affected by the soil moisture regime and genotypes. Furthermore, field evaluation is necessary to confirm soil enzymes activities in soil and P nutrition of soybean to assess practical utility of these bacteria.

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