Scientific Papers. Series A. Agronomy, Vol. LVII, 2014
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

# THE EFFECTS OF PHOSPHORUS DEFICIENCY AND RHIZOBACTERIA ON PHOSPHORUS CONTENTS OF TWO SOYBEAN (*Glycine max* L.) CULTIVARS GROWN AT LOW WATER SUPPLY

**Vladimir ROTARU[1], Simion TOMA[1], Ana BIRSAN[2]**

[1]Institute of Genetics, Physiology and Plant Protection, Moldavian Academy of Sciences
20 Padurii str., MD 2002, Chisinau, Republic of Moldova
[2]State University of Moldova, 60 Mateevici str., MD 2009, Chisinau, Republic of Moldova

Corresponding author email: rotaruvlad@yahoo.com

***Abstract***

*Phosphorus (P) deficiency and low water supply are major environmental constraints* for *agricultural production in many regions. Compared to cereal crops, the soybean (Glycine max.L.) is more susceptible to phosphorus insufficiency and drought. A soil pot experiment was conducted in the greenhouse to investigate the effects of phosphorus deficiency and pseudomonas florescence and azotobacter chroococcum strains on phosphorus uptake by soybean grown under temporary drought conditions. Soybean cultivars Zodiac and Horboveanca were grown on soil-sand mixture with P deficiency at two water regimes - 70% water holding capacity of soil (WHC) and 35% WHC. Plants were harvested and analyzed for P contents in each organ after 12 days of water deficit at the setting pod stage. Phosphorus deficiency significantly decreased nutrient uptake by all plant parts especially in drought conditions. Leaves have shown a higher sensitivity to P deficiency than roots. Application of rhizobacteria induced P uptake in both cultivars but their effects were more pronounced in Horboveanca under both well watered and dry soil conditions. Phosphorus fertilization alone of plants significantly increased P accumulation irrespective of soil moisture regime and Horboveanca displayed a higher response than Zodiac cultivar. The P accumulation in the roots of both cultivars and in the pods of Zodiac enhanced after the application of bacteria or P fertilizer under temporary drought. Interactive effects of applied bacterial strains and water soil regime depend on the cultivar used. From the results of the present experiment it can be concluded that biofertilizers seem to be particularly beneficial to P uptake in normal as well as in water limiting environment.*

***Key words***: *drought, phosphorus*, *rhizobacteria, soybean.*

## INTRODUCTION

Phosphorus (P) is an essential nutrient required for plant growth, development and productivity, but its low availability in soils makes it one of the least mobile mineral elements. Phosphorus deficiency is worldwide spread and it affects agriculture sustainability in many agricultural regions. In field conditions, low soil fertility frequently is accompanied by droughts. Drought and low P availability in soil presents a serious problem to agriculture production on global and national levels. Chemical P fertilizers are the main source of plant available phosphates alleviation in agroecosystems, but the majority (approximately 75-80%) of applied phosphates are fixed in soil and they became less available for crops (Vance et al., 2003). Nowadays, the use of P fertilizer is reduced substantially in many countries because of the higher costs of these fertilizers and many farmers don't have the capacity to buy them. In addition, the long-term sustainability of fertilizers application is questionable because according to some estimates the world reserves of rock phosphates are expected to be exhausted in the next 50 years at current rates of consumption (Gilbert, 2009).

Under low levels of soil moisture, not only water but also nutrient availability may be severely decreased when compared with well watered conditions (Gahoonia et al., 1994). There is need to identify suitable biological approach to improve plant nutrition under poor soil resources such as scarce availability of phosphates and water.

Plant growth promotion rhizobacteria (PGPR) due to their ability to increase nutrient uptake and thus to promote plant growth, have often been suggested as a means to improve crop production (Adesemoye et al., 2009). There are

evidences that the application of P-mobilizing microorganisms is regarded to be an alternative strategy to improve the supply of P to agricultural crops (Rodrigues and Fraga, 1999).
Whitelaw (2000) stated that the bacterial genera *Pseudomonas* are the most powerful P solubilizer. Simulative effects on plant P uptake under P deficient conditions were found after the application of *Pseudomonas* strains in a pot experiment with cotton and pea (Egamberdiyeva and Höflich, 2004).
Plant growth promoting bacteria could effectively colonize plant roots and provide plant growth through different mechanisms that include enhancing mobilization of less available nutrients and subsequent increase in nutrient uptake (Dey et al., 2004). There are many experimental results which demonstrated the beneficial effects of PGPR on nutrient acquisition and plant growth. Their analyses have shown that the application of PGPR is a suitable approach for reducing the doses of chemical fertilizers and their pollution hazards that could also support eco-friendly crop production (Dey et al., 2004).
Legumes are well known to be an essential component of various agroecosystems in order to develop organic agriculture. Legumes, particularly soybean, have a higher demand of P nutrition because nitrogen fixation required considerable sources of energy. This species is sensitive to low P supply and drought.
We hypothesized that particularly under unfavorable conditions P deficiency and low water supply the application of rhizosphere microorganisms would improve plant growth by increasing phosphorus absorption in plant parts.
Moreover, the analyses of available literature data revealed that the effects of PGPR on nutrient uptake were examined as a rule under normal water conditions and there is scarce information regarding their impact on P uptake by legumes under combined abiotic stresses the low P and water deficit. Suspension of nitrogen-fixing *(Azotobacter chroococcum*) and phosphate solubilizing (*Pseudomonas florescence*) bacteria (Rh - in Figures) was used as tested strains in the study and soybean plants which have a higher demand of P nutrition.
Thus, the main objective of the research was to investigate the influence of phosphorus deficiency and *pseudomonas florescence and azotobacter chroococcum* suspension application on P accumulation within soybean organs of two cultivars were either cultivated under normal or suboptimal soil water regime.

## MATERIALS AND METHODS

To accomplish the objective of this study it was conducted out a pot experiment in a greenhouse under controlled humidity conditions. The research included two soybean (*Glycine max., L.*) cultivars: Zodiac and Horboveanca that differ by potential productivity and responsiveness to phosphorus fertilization. The soil was chernoziom carbonated with low level of available phosphates, basic pH (7,7), which was mixed with sand in a 3:1 ratio (by volume). Seed inoculation with bacterial preparation was carried out on the basis of *rhizobium japonicum*. Phosphorus as monocalcium phosphate dose of 100 mg $kg^{-1}$ was administered to the soil (P100), being regarded as sufficiently supplied with phosphorus and control treatment (without chemical and biofertilizers, P0) - deficient in phosphorus. The bacterial suspension of *azotobacter chroococcum* and *pseudomonas fluorescens* strains (equivalent to $10^8$ cells $ml^{-1}$) was applied to the soil before sowing. Soil moisture regime was achieved by watering to 70% of the water holding capacity of the soil (WHC) in the control as optimal value and 35% WHC treatment option, being considered as water stress for soybean. The pots were distributed in a complete randomized block design with 3 replications and three plants per pot. Temporary drought regime began in the full-flowering stage and lasted 12 days. The plants were harvested at the end of water stress and separated into roots, leaves, stems, nodules and pods and dried at 60ºC for 3 days. Dry weight of each fraction was measured. The concentration of P was measured in samples of ground tissues following wet digestion, using the phosphovanado-molybdate method of Murthy and Riley (1962). The phosphorus contents in plant parts were calculated by multiplying dry weight of each organ with P concentration.
Data in figures represent the average value of the results of chemical analysis of plants in

three replications. The experimental results were analyzed statistically, determining significant differences at the level of P = 0.05. In figures are presented means of 3 replicates with standard error.

## RESULTS AND DISCUSSIONS

Plant growth usually benefits from application of rhizobacteria in nutrient-deficient soils. It is well known that PGPR can improve the nutrient status of their host plants (Rodrigues and Fraga, 1999).

One of the main objectives of our research was to explore the beneficial effects of potential bacterial inoculants (nitrogen fixer and phosphate solubilizer) that can enhance mineral nutrition of plants. In our study, phosphorus fertilization and PGPR application generally increased plant growth and P uptake of both soybean cultivars in soil with initial P deficiency. The data in Figures 1-4 show that the accumulation of phosphorus in plant parts varied among cultivars and were significantly altered by rhizobactria (Rh) application and soil water regime.

In P deficiency treatment the total plant uptake of P was generally higher for Horboveanca compared with Zodiac under optimal moisture level (70% WHC). However, there was no significant difference between cultivars when plants were subjected to limited water conditions (35% WHC). Consequently, the water suboptimal regime reduced substantially P accumulation in Horboveanca than in Zodiac. Phosphorus accumulation in leaves of the P deficiency and bacteria application treatments did not differ significantly while the P sufficiency (P100) treatment increased evidently the uptake of nutrient in Zodiac (Figure 1 and 2). The analysis of experimental results revealed that the administration of biofertilizers improved nutrient contents in the stems of Zodiac. Drought is considered one of the major environmental abiotic factors with a considerable adverse influence on the plant performance, in particular on the nutrient status of crops. In our study it was revealed that low water supply significantly reduced P contents in both cultivars irrespective of treatments. There is evidence that improving P nutrition of plants could attenuate the negative impact of droughts on plant growth and development. As we expected, the phosphorus sufficient treatment (P100) had positive impact on P uptake in all parts of soybean plants. The P concentrations of roots in the control treatment and with biofertilizers administration were similar in Zodiac cultivar under drought but rhizobacteria increased this trait by 14.5% in normal soil moisture level.

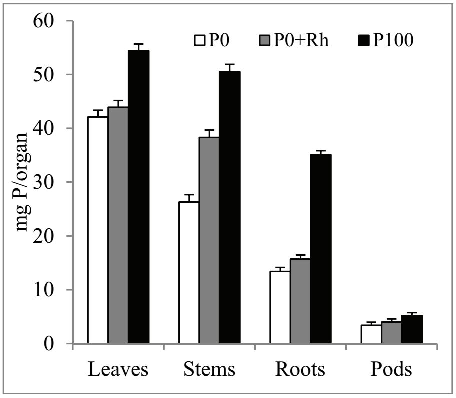


Figure 1. Effects of P deficiency and rhizobacteria (Rh) application on P content of cultivar Zodiac grown in normal water conditions (70% WHC)

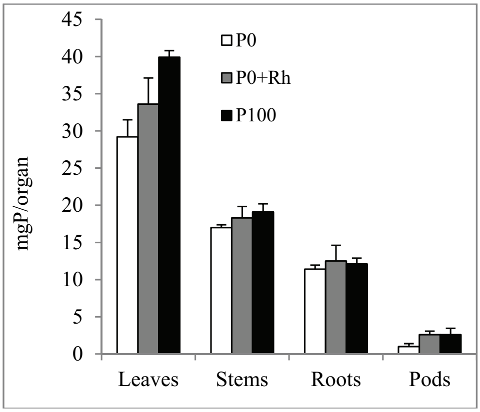


Figure 2. Effects of P deficiency and rhizobacteria (Rh) application on P content of cultivar Zodiac grown in suboptimal water conditions (35% WHC)

This was linked with more biomass accumulation by roots in no water stress conditions (data are not presented). Compared to the P-deficiency plants the P content of pods in the treatments with biofertilizers and chemical P fertilizer was approximately twice as high at low water supply (Figure 2). Wani and collaborators (2007) revealed a beneficial impact of PSB and $N_2$-fixing bacteria on chickpea plants with a significant increase in productivity and uptake of phosphorus and nitrogen.

Likewise, Li and coworkers (2013) demonstrated an increase of $N_2$ fixation in inoculated alfalfa with phosphate solubilizing rhizobium and nitrogen fixing bacteria when both nitrogen and phosphorus are limited. It may be concluded that the application of rhizobacteria increases significantly P uptake in the stems of Zodiac in normal water conditions but their influence was not registered in roots in water deficit treatment. Probably, these results could be explained by the decreased transport of nutrients from roots to above ground plant parts at low water supply. Our results are consistent with experimental data obtained in cowpea (Franzini et al., 2010; Neuman and George, 2009).

In normal water soil conditions the differences between treatments with P deficiency and the application of PGPR were not so pronounced in terms of P contents in leaves. In contrast, under dry soil conditions P deficiency and biofertilizers administration treatments were significantly different in biomass production (data not presented) and total plant P content. Thus, soil dryness limited P availability in the P deficiency-plants, compared with the biofertilizers treatment. The similar effects were registered in maize (Krey et al., 2011) and rice (Panhwar et al., 2011). Interesting data were obtained in Horboveanca cultivar which is considered more responsive to supplemental P nutrition than Zodiac. The results registered by this cultivar indicate that the suspension application of *pseudomonas florescence* and *azotobacter chroococum* contributed significantly to increase plant P uptake and plant biomass production (Figure 3 and 4).

At the optimal soil moisture level, as shown in Figure 3, there were significantly higher P contents in stems, roots and pods of treatments with P and biofertilizers application compared with those of the control plants. However, there was poor difference in terms of P accumulation in leaves between P deficiency and microorganism treatments. Experimental results demonstrated that the administration of bacterial suspension induced a significant increase of P uptake in stems and roots of 7.5% and 32.1% in comparison to control plants (without rhizobia supply), when plants were not subjected to low water regime. The highest increase in phosphorus accumulation was observed in the pods of plants grown with *pseudomonas florescence and azotobacter chroococcum* biofertilization under well watered conditions. However, the application of these strains had no significant effects on the accumulation of nutrient in the pods under water limited conditions (Figure 4).

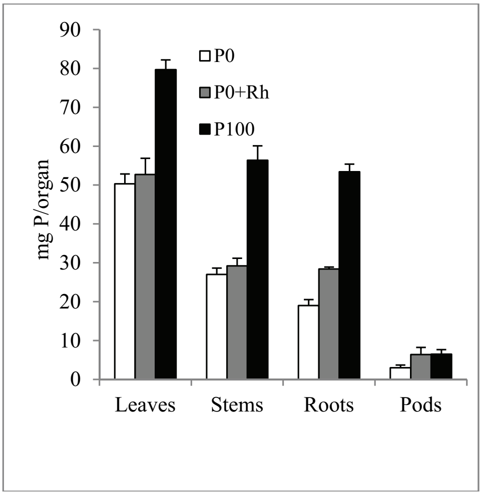


Figure 3. Effects of P deficiency and rhizobacteria (Rh) application on P content of cultivar Horboveanca grown in normal water conditions (70% WHC)

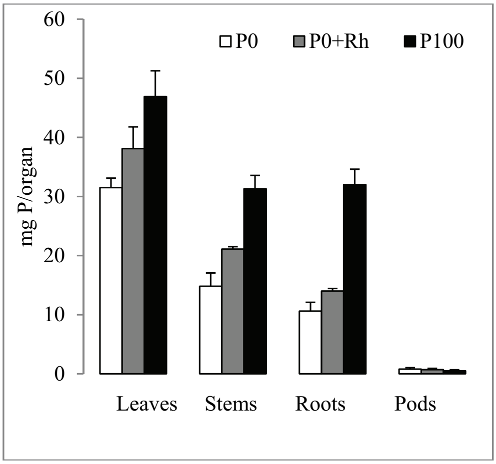


Figure 4. Effects of P deficiency and rhizobacteria (Rh) application on P content of cultivar Horboveanca grown in suboptimal water conditions (35% WHC)

In contrast, at the suboptimal soil moisture regime, significant differences between treatments were recorded, regarding phosphorus contents in leaves, stems and roots. Thus, the biofertilizers and mineral phosphorus applications contributed to an increase in total nutrient uptake of 19.4% and 47.9% in plants for Horboveanca under suboptimal water conditions, respectively. As related in literature the rhizobacteria application improved P availability in soil from hard compounds of organic form. In addition, they in association with plant roots could stimulate protons and low weight organic acids release that in turn improve the nutrient condition of plants (Rodrigues and Fraga, 1999). Thus, the lowest phosphorus accumulation in plant parts was obtained in control plants of both cultivars, particularly in water limiting environment.

The highest phosphorus accumulation in the present work was obtained in the plants fertilized with P for Zodiac and Horboveanca.

Hence, considering the influence of the PGPR application on this parameter, the effect was lower than those of the industrial fertilizer. There is a body of reports on plant growth promotion by bacteria (Kery et al., 2011) that have the ability to enhance P availability for plants. The use of PGPR can improve phosphorus uptake by the plant which in turn would result in more energy available for nitrogen fixation by rhizobium.

In addition, inoculation with PGPR improves plant growth parameters and nutrient uptake, but this effect was more pronounced in Horboveanca than in Zodiac, expressing that the colonization of rhizobacteria also significantly reduced the negative effect of P deficiency (Figures 1-4). The uptake of other essential micronutrients from the soil due to biofertilizers could play a role in general plant growth improvement as well as in more indirect effects upon the $N_2$-fixing system. The obtained results indicated that bacteria application increased the phosphorus content in roots and pods, compared with their controls which were not supplied with rhizobacteria. These findings are in agreement with that of Xiurong et al. (2011) and Li et al. (2013). Increased mineral nutrient status in roots would contribute to enhance the acquisition of scarce resources from dry soil in our case of water and mineral nutrients. Also, they could lead to increase photosynthesis, making a greater proportion of photosynthates available to plant - rhizobium symbiosis (Mortimer et al., 2008).

In summary, our results have evaluated the effects of rhizobacteria application and P fertilizer on low soil fertility on the soybean phosphorus uptake under suboptimal soil moisture regime. These investigations show a potential strategy of microorganisms used for agricultural development in marginal lands that are often deficient in P.

Likewise, these findings point to a higher dependency of Horboveanca, cultivar responsive to supplemental P nutrition, compared with Zodiac on the bacterial application for growth and nutrient uptake under conditions of drought and/or low nutrient availability. In this study we used non-sterile soil due to the fact that in many researches, plant growth promotion with PGPRs was observed usually under controlled conditions where these bacteria do not have to compete with a range of indigenous soil microbes. At present, unfortunately, the use of microorganisms in the biotechnology of legume production is very modest. Although the effects of rhizobacteria symbiosis are more difficult to reveal in the field there is a need to examine their functional properties in further studies.

## CONCLUSIONS

Phosphorus deficiency decreased the phosphorus contents in soybean organs in particular under water-limited conditions.

In P-deficient soils, application of *P. fluorescens* and *A. chroococcum* strains can affect the P supply of soybean plants. However, the magnitude of this influence is affected by soil water regime.

The application of rhizobacteria biofertilizers led to a significant increase in total phosphorus uptake by Zodiac and Horboveanca soybean cultivars. Cultivar Horboveanca displayed a higher response to PGPR application than Zodiac. Subsequent field experiments should be consider the agronomic effects of microorganisms in combination with different doses of phosphorus fertilizer and organic manure on crop production.

## AKNOWLEDGEMENTS

We are grateful to the Supreme Council of Research and Development of the Moldavian Academy of Sciences of the Republic of Moldova for the financial support to conduct this study.

## REFERENCES

Adesemoye A.O., Torbert H.A., Kloepper J.W., 2009. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb. Ecol. 58, p. 921-929.

Dey R., Pal K.K., Bhatt D.M., Chauhan S.M., 2004. Growth promotion and yield enhancement of peanut (Arachis hypogaea L.) by application of plant growth-promoting rhizobacteria. Microbial Res. 159, p. 371-394.

Gahoonia T.S., Raza S., Nielsen N.E., 1994. Phosphorus depletion in the rhizosphere as influenced by soil moisture. Plant Soil 159, 1, p. 213-218.

Egamberdiyeva D., Höflich G., 2004. Importance of plant growth promotion bacteria on growth and nutrient uptake of cotton and pea in semi-arid regions Uzbekistan. J. Arid. Env. 56, p. 293-301.

Franzini V.I., Azcor N.R., Latanze-Mendes F., Aroca, R., 2010. Interaction between Glomus species and Rhizobium strains affect the nutritional physiology of drought stressed legume hosts. J. Plant Physiol. 167, p. 614-619.

Gilbert N., 2009. The Disappearing Nutrient. Nature, 461, p. 716-718.

Krey T., Maria Caus, Christel B., Silke Ruppel, Bettina Eichler-Löbermann., 2011. Interactive effects of plant growth–promoting rhizobacteria and organic fertilization on P nutrition of *Zea mays* L. and *Brassica napus* L. *J.* Plant Nutr. Soil Sci. 174, p. 602-613.

Li J.F., Zhang S.Q., Huo P., Shi S. Miao Y. Y., 2013. Effect of phosphates solubilizing rhizobium and nitrogen fixing bacteria on growth of alfalfa seedlings under P and N deficient conditions. Pak. J. Bot. 45, 5, p. 1557-1562.

Mineev V.G., 1989. Practicum de agrochimie. M., p. 389.

Mortimer P.E., Perez-Fernandez M.A., Valentine A.J., 2008. The role of arbuscular mycorrhizal colonization in the carbon and nutrient economy of the tripartite symbiosis with nodulated *Phaseolus vulgaris*. Soil Biol. Biochem., 40, p. 1019-1027.

Murthy J., Riley J.P., 1962. A modified single solution method for the determination of phosphate in natural water. Anal. Chem, 27, p. 31-36.

Panhwar Q.A., O. Radziah, A. Zaharah Rahman, M. Sariah, I. Mohd Razi, Naher U.A., 2011. Contribution of phosphate-solubilizing bacteria in phosphorus bioavailability and growth enhancement of aerobic rice. Span. J. Agr. Res. 9, 3, p. 810-820.

Rodríguez H., Fraga R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances 17, p. 319–339.

Vance C.P., Uhde-Stone C., Allan D.L., 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytologist 157, p. 423-447.

Xiurong W., Qiang P., Fengxian C., Xiaolong Y., Hong L., 2011. Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. Mycorrhiza 21, p. 173–181.

Wani P.A., Khan M.S., Zaidi A., 2000. Impact of heavy metal toxicity on plant growth, symbiosis, seed yield and nitrogen and metal uptake in chickpea. Aus. J. Exp. Agric. 47, p. 712–720.

Whitelaw M.A., 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. Adv. Agronomy, p. 69.