THE INFLUENCE OF RHIZOBACTERIA AND PHOSPHORUS SUPPLY ON NITROGEN AND PHOSPHORUS CONTENTS IN SOYBEAN UNDER INSUFFICIENCY MOISTURE OF SOIL

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Abstract

Rhisophere bacteria have a beneficial impact on plant growth and development due to improvement of mineral nutrition. In order to elucidate the effect of *azotobacter chroococcum* and *pseudomonas fluorescence* on nitrogen and total and inorganic phosphorus concentrations in plants a pot experiment was carried out. Soybean (Glycine max. L) plants were cultivated on soil-sand mixture. A set of plants was subjected to water stress conditions of 35% WHC (water holding capacity) at flowering stage and other one grown under normal moisture of soil, 70% WHC. Experimental results revealed that the application of phosphorus alone or in combination with rhizobacteria did not change the concentration of nitrogen in leaves, but it was observed an increase of nitrogen contents in roots. The same trend was demonstrated in pattern of total phosphorus concentration in soybean parts. The utilization of suspension of microorganisms and phosphorus increased significantly the concentration of inorganic phosphorus under normal as well as under insufficient moisture level. Thus, biofertilizer application could be considered as a strategy to attenuate negative effect of drought through stimulation of nutrients contents in soybean.

Key words: *Glycine max*., nitrogen, phosphorus, rhizobacteria, water stress

Soybean is one of the protein and oil essential sources for human and livestock feed. Soybean plants due to their good capacity of nitrogen fixation improve soil fertility and reduce doses of industrial fertilizers.

According to literature data this species is very susceptible to drought and low phosphates in soil (Wang X et al., 2010). However, most of the soils in Republic of Moldova as well as at global scale are characterized by low level of phosphorus availability (Andries S., 2007). A large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants (Yadav K., Dadarwal H., 1997). Likewise, reserves of rock- phosphorus are finite with an estimated depletion of sources expected to occur within the next 50–60 years (Vance C et al., 2003). It must be emphasized that the higher cost of phosphoric fertilizer became a limiting factor for crop production. Several plant growth-promoting rhizobacteria (PGPR) have shown potential to enhance phosphorus solublization and nutrition of crops (Adesemoye A., Kloepper J., 2009, Krey T et al., 2011**)**. The application of rhizobacteria in soybean biotechnology could have a promising benefit for plant nutrition, partially overcome P deficiency. Thus, in the context of increasing international concern for food and environmental quality, the use of PGPR for reducing chemical inputs in agriculture is a potentially important issue.

PGPR have been applied to various crops to enhance growth, seed emergence and crop yield, and some have been commercialized (Dey R et al., 2004). Rhizosphere bacteria can affect plant growth through different mechanisms such as nitrogen fixation, production of plant growth regulators (Vessey K., 2003) and increasing plant water and nutrient uptake (Dey R et al., 2004, Rodrigues H., Fraga R., 1999). The ability of *Pseudomonas* strains to increase solubility of phosphate sources and non-absorbent organic phosphate emphasizes the need of using them to increase the absorbing of nutrients, especially phosphorus, in terms of nutrient shortages (De Freitas J et al., 1997).

The objective of the research was to investigate under greenhouse conditions the influence of P and bacteria *azotobacter chroococcum* and *pseudomonas fluorescence* on the nitrogen, total and inorganic phosphorus contents in soybean under suboptimal moisture regime of soil.

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MATERIALS AND METHODS

In order to accomplish the research objective it was carried out a factorial pot experiment under semi controlled environmental conditions with soybean (*Glycine max. L* cultivar Zodiac). The phosphorus deficiency soil was represented by cernoziom carbonated and was mixed with sand (1:1 v/v). The treatments included variants with suspension application of *azotobacter chroococcum* and *pseudomonas fluorescence* in soil without (P0) or with of phosphorus (100 mg P per kg of soil). Three plants were grown in each pot. There were four replications of treatment. The soybean seeds were inoculated with bacteria strain *Bradyrhizobium japonicum*. Plants were cultivated at two water soil regime 70% WHC (water holding capacity) as normal level and 35% WHC as suboptimal level. Soil water shortage was imposed at the flowering stage for two weeks. Plants were cut at ground level separated from roots, nodules and dried at 60 ºC for 72 hours. These samples were finely ground and analyzed for nitrogen content by a Kjeldahl procedure and total and inorganic P determined by vanadate-molybdenum blue method (Murthy J., Riely J., 1962).

RESULTS AND DISCUSSION

The bacterial species *azotobacter chroococcum* and *pseudomonas fluorescence* as well as phosphorus supply caused significant increase of dry weight of roots and shoots of soybean and the results have been reported (Rotaru V., 2012). The nutritional status of plants was evaluated by assay of nitrogen, total and inorganic phosphorus contents in leaves and roots (tab. 1, fig. 1, 2). Under optimal water conditions the nitrogen concentration in leaves was higher of aninoculated plants compared to inoculated plants.

Table 1

Changes in nitrogen concentration (%, dry weight) in leaves and roots of soybean in relation to rizosferic bacteria and P supply under water stress conditions

Treatments	Leaves		Roots	
	70% WHC	35% WHC	70% WHC	35% WHC
A week after water stress				
P0	5,13	4,77	3,25	3,44
P20	4,84	4,66	2,77	2,86
P100	4,83	4,52	2,83	2,93
P0+MO	4,88	4,66	3,05	3,04
P100+MO	4,64	4,96	3,02	3,18
Two weeks after water stress				
P0	4,61	4,51	2,95	2,80
P20	4,44	4,44	2,79	2,87
P100	4,33	4,49	2,76	2,76
P0+MO	4,19	4,49	2,77	2,80
P100+MO	4,14	4,46	2,81	2,97

Experimental results established an increase of nitrogen content in control roots compared to the plants with fertilization. Perhaps this result obtained in treatment without phosphorus application is due to biological dilution effect. The combined supply of microorganisms (MO) and phosphorus (P100 mg kg soil) increased nitrogen content in leaves by 6% of water stressed plants. The trend effect of rhizobacteria was not affected by soil moisture level. Likewise, combined application of microorganisms and fertilizer increased the concentration of nitrogen in roots by 8% (tab. 1). The data analysis of the second harvest of plants din not revealed any significant changes in distribution of total nitrogen in leaves. Under optimal moisture of soil phosphorus supplemental nutrition separately or in combination with MO decreased the nutrient concentration. However, in water-stressed plants the concentration of N in roots at the second harvest was increased by 7,1% due to both bacterial inoculation and P supply. Our results are consistent with the data obtained by Arnon I., (1975) that have been demonstrated an increase of nitrogen in corn plants under water stress conditions.

Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants.
The growth conditions did not affect the concentration of total phosphorus in leaves

tissues. The experimental results shown weak modifications in leaves after bacteria

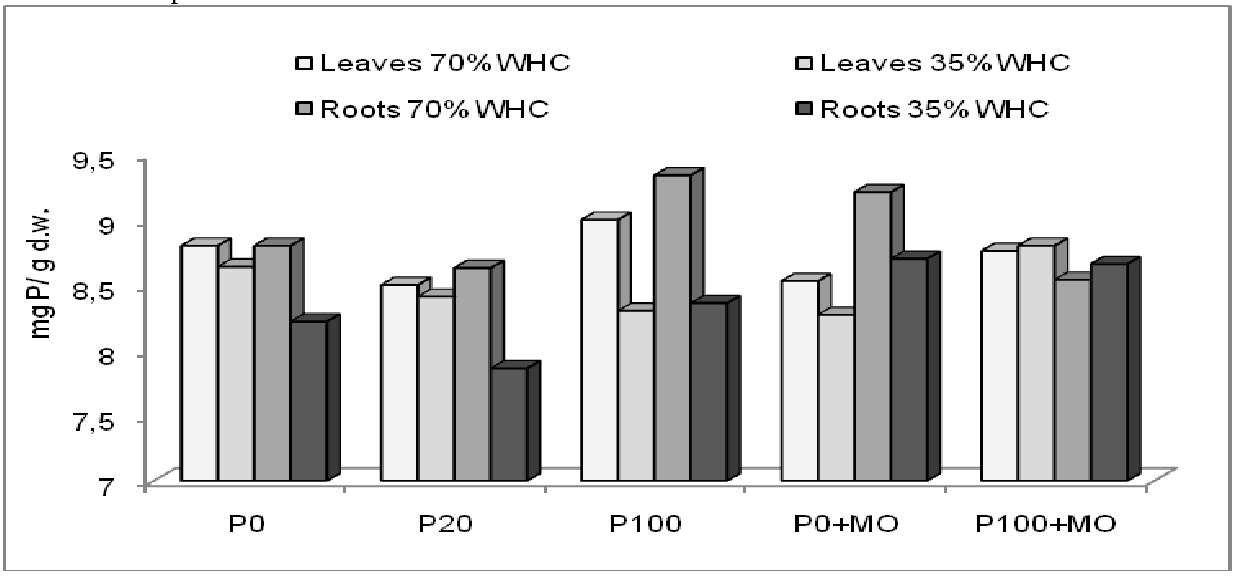

Fig. 1. **Concentration of total P in leaves and roots in relation to supplemental P nutrition and rizosferic bacteria application under suboptimal soil conditions (a week water stress**)

supply. However, it was observed an accumulation of phosphorus in roots in treatment of bacteria application without phosphorus supplemental nutrition of normal hydrated plants (fig. 1). The same trend was found after 8 weeks of plant growth (data are not presented). In treatment with MO application on native soil (P0) the content of P decreased from 9,9 to 7,7 mg P dry mass of leaves compared to reference treatment. Also, it was demonstrated that the nutrient concentration in roots increased with increasing the dose of phosphorus under suboptimal moisture of soil Thus, the soil amendment with biofertilizer or P supply alleviated partly phosphorus nutrition of plants under drought conditions.

An important character of phosphorus metabolism is considered the content of inorganic P in plant tissues.

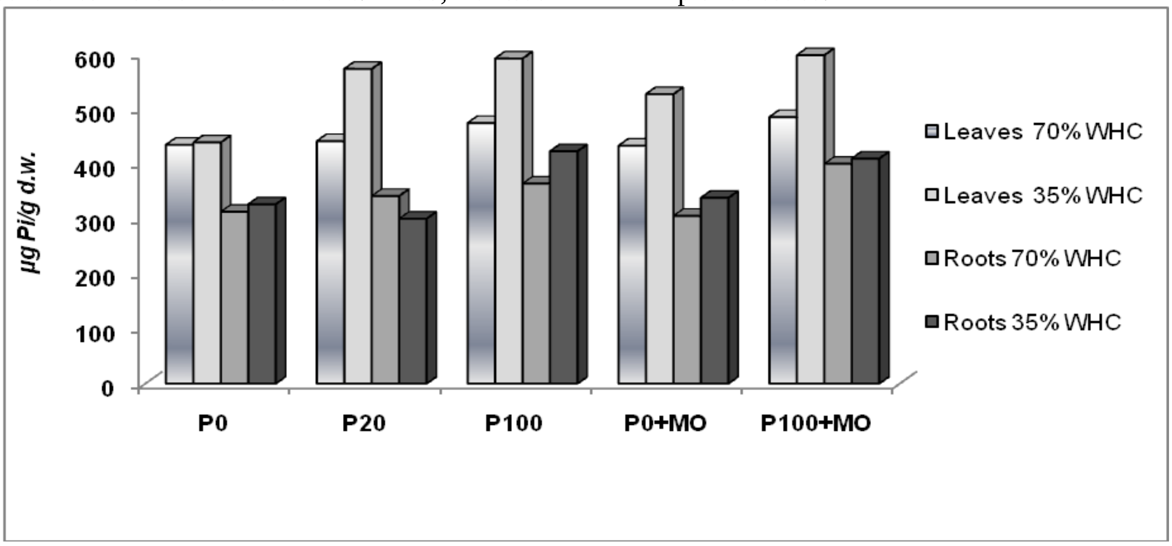

Fig. 2. **Concentration of inorganic P in leaves and roots in relation to supplemental P nutrition and rizosferic bacteria application under suboptimal soil conditions**

Supplemental mineral nutrition contributed to modification of Pi contents in leaves and roots. The determination of this physiological parameter demonstrated that the insufficiency of soil moisture increased its concentrations in vegetative organs of plants (fig. 2). Its content in both parts increased due to alone application of P to stress plants (35% WHC). At the second term of plant harvest it was observed an increase of Pi concentration in leaves in treatment with MO application under normal moisture conditions. Our results are in accordance with the previous studies indicating increase P accumulation in *Phaseolus vulgaris* due to P application and PGPR inoculation (Heckman R., Kamprath E., 1995; Olivera M et al., 2004). The data of present investigation show that the administration of phosphorus induced the accumulation of Pi in leaves especially under suboptimal water regime of soil. The use of MO together with P decreased the level of this parameter in leaves irrespective of soil moisture level. But its concentration under limited water conditions increased in leaves with sufficiently P supply (P100). It should be noted that the application of microorganisms did not change the nutrient content under normal moisture but it was observed more pronounced beneficial effect under water stress conditions. An increased P uptake of 16% was observed for soybean after the application of the mixture p*seudomonas fluorescence* and *azotobacter chroococcum* in the treatment without fertilization (fig. 2). The higher P concentration in the plant benefits the bacterial symbiont and the functioning of its nitrogenase leading to increase N fixation, which in turn promotes root development (Lee K., Ponhurst C., 1992). Hence, supplemental P nutrition as well as MO administration had a beneficial effect upon the absorption phosphorus in roots. For instance, under optimal soil moisture it was demonstrated an accumulation of Pi in tissues of roots due to application of phosphorus alone or in combination with MO.

Thus, our research established that phosphate insufficiency in cernoziom carbonated decreased the concentration of phosphorus inorganic in roots and leaves irrespective of soil moisture regime. Taken together, results suggest that rhizobacteria are able to increase the mineral nutrition, thereby improving the growth of plants. The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers for sustainable soybean cultivation in low fertility of soil. Further investigations, including efficiency test under field conditions, are needed to clarify the role of rizosferic bacteria as biofertilizers that exert beneficial effects on grain productivity of soybean.

CONCLUSIONS

Low availability of phosphates in cernoziom carbonated decreased the content of inorganic of phosphorus in roots and leaves of soybean irrespective of water soil regime.

Our results suggest that soybean plants benefit from the bacterial application of *Pseudomonas fluorescens* şi *Azotobacter chrooccocum* under dry soil conditions mainly in terms of an improved mineral nutrition.

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