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EFFECT OF *Bradyrhizobium japonicum* AND *Pseudomonas putida* APPLICATION ON GROWTH AND YIELD OF SOYBEAN UNDER PHOSPHORUS AND WATER DEFICIENT CONDITIONS

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Abstract

In recent years, the use of soil microorganisms has generated great interest in crop production, by reducing the damage from drought stress and improving nutrition, hence, providing alternative solutions for sustainable agriculture. A pot experiment was conducted to examine the impact of two rhizobacteria Bradyrhizobium japonicum and Pseudomonas putida on growth and grain productivity of soybean (Glycine max (L.) Merr.*) under phosphorus insufficiency and moderate drought conditions. Soybean seeds were inoculated with Bradyrhizobium japonicum and Pseudomonas putida was administered in soil before the sowing. Soybean plants were grown in a soil: sand mixture with low phosphorus supply. Plants were treated with two regims of irrigation water, i.e., 70% of water holding soil capacity (WHC) and 35% of WHC as moderate drought. The water deficit was imposed at the flowering stage for 12 days. Plant growth traits and soybean yields were significantly altered by low P supply, water stress and PGPR treatments. P insufficiency and temporary drought had a sinergetic negative effect on soybean performance. The results of this experiment revealed significant positive effects of Rhizobium inoculation and Pseudomonas putida on growth, nodulation and yield of plants under both water regimes but drought reduced their beneficial impact. Experimental data demonstrated that integrated application of nitrogen-fixer bacteria and P. putida better improved the growth and plant productivity as compared to the application of B. japonicum alone. In conclusion, the combined utilization of B. japonicum and P. putida can mitigate deleterious effects of temporary drought stress in soybean under phosphorus and water deficiency conditions.*

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

INTRODUCTION

In the last two decades, the use of chemical phosphorus (P) fertilizers in crop systems has decreased in many countries, particularly in countries with vulnerable resources of rock phosphates, due to their higher price. Beneficial free-living bacteria, referred to as plant growth promotion rhizobacteria (PGPR) are components of the plant rhizosphere and have been found in association with many plant species including leguminous plants (Majeed et al., 2015). The exploitation of rhizobacteria is an alternative of industrial amendments and has a considerable importance for crop production (Yadav et al., 2013). Among the various microorganisms with potential to enhance plant growth, the role of biofertilizers such as *Rhizobium* and *Pseudomonas* in tolerating environmental stress has been well established and has received increased attention from researchers (Kadian et al., 2013). PGPR can enhance plant growth directly by providing plants with nutrients such as nitrogen via nitrogen fixation or by supplying phosphorus from soil bound phosphate due to the activities of soil microorganisms (Berg, 2009; Yadav and Dadarwal, 1997). Likewise, these microorganisms are known for their ability to synthesize several plant growth hormones such as auxins, cytokines and others (Berg, 2009; Yadav et al., 2013).

Drought and low phosphorus supply are the major abiotic factors which are responsible for reducing the production of soybean in semi-arid regions of the world. The soybean (*Glycine* *max.* L) is a member of the family *Leguminosae*, mainly cultivated for oil production as well as for animal fodder. Soybean plants are sensitive to phosphorus deficiency and drought. Adequate phosphorus nutrition has fundamental physiological and biochemical roles, influencing the water economy and plant growth, affecting water uptake, root growth, transpiration and stomatal regulation (Singh et al., 1997). Many studies have documented the role of *Bradyrhizobium* inoculation in improving the growth and yield of soybean (Egamberdiyeva et al., 2004; Israel, 1993). The research of Tilak et al. (2006) concluded that growth, nodulation and enzymes activity were significantly increased in plants co-inoculated with *Pseudomonas putida*, *P. fluorecens* and *Bacillus cereus*, compared with those inoculated only with *Rhizobium.* However, there are limited studies referring to the utilization of *Bradyrhizobium japonicum* together with other genera such as *Pseudomonas putida.* It is necessary to note their effects on crops have been studied under favorable soil moisture conditions, as a rule, and little is known about their interactions under multiple environmental stresses. Therefore, the present investigation was undertaken to evaluate the effects of seed pre-treatment with *B. japonicum* alone or in combination with the application of *Pseudomonas putida* under insufficiency or adequate P supply of soybean plants subjected to moderate drought conditions.

MATERIALS AND METHODS

To evaluate the effect of *B. japonicum* and *P. putida* inoculation on the growth and yield of soybean plants under drought stress a pot experiment was conducted in a greenhouse. The soil of carbonated cernoziom with low phosphate availability was mixed with sand in a proportion of 3:1 (soil: sand). The seeds were treated pre-sowing with the suspension of *Bradyrhizobium japonicum* of local strain 646. For rhizosphere inoculation, live cultures *Pseudomonas putida* were added into the soil before the sowing of the seeds. These rhizobacteria were tested without fertilization (low P supply - P0) and on soil fertilized with potassium phosphate at a rate of 100 mg P kg^{-1} soil (P100). The experiment was laid out in a randomized complete block design, with eight replicas of each treatment. The study was carried out using Horboveanca cultivar of soybean sensitive to drought. Soybean seeds were surface-sterilized by washing them with 96% ethanol for 30 seconds and 2.5% sodium hypochlorite for 3 minutes, and then rinsed several times with sterile, distilled water. In each pot, six healthy and uniform seeds of soybean were sown at a depth of 3 cm. After complete germination, the plants were thinned to two plants per pot. Half of the pots were well watered throughout the experiment (70% WHC) while half was subjected to water stress conditions by reducing the irrigation rate (35% WHC) at the flowering stage. Plant drought of moderate stress was imposed for 12 days. A set of plants was carefully harvested at the beginning of the pod setting stage to study the growth, root development and nodulation potential of soybean while the other set of plants was harvested at R8 stage to determine grain productivity. After the harvest, roots and shoots were weighed separately to determine fresh weight, and then placed in an oven to dry at 60°C until a constant dry weight was obtained. Data were subjected to varying means off analysis and categorized using the “least significant difference” test in the Statistic program 7.

RESULTS AND DISCUSSIONS

The main parameter for evaluation of rhizobacteria and chemical fertilizer effect in crop cultivation is considered the accumulation of dry matter. The experimental results have shown that plant growth displayed significant responses to biofertilizer application as well as to P fertilization. Biomass production in all treatments with PGPR of soybean increased significantly in comparison to uninoculated plants (Figures 1 and 2). According to the results, root biomass was also found to be increased significantly irrespective of treatments over control (data not shown). The least accumulation of dry matter was recodered in treatment with insufficiency of P and water deficit. Experimental data found that the dry weights of plants increased by 13.1% after *B. japonicum* inoculation under normal soil moisture (Figure 1). Similarly, the growth promotion of soybean by inoculation with *Bradyrhizobium* was reported by Rahmani and Saleh-Rastin (2001) in P-deficient soil. The best increase was recorded in the treatment of the combined application of *B. japonicum* and *P. putida* in comparison with the single inoculation of nitrogen-fixer rhizobacteria.

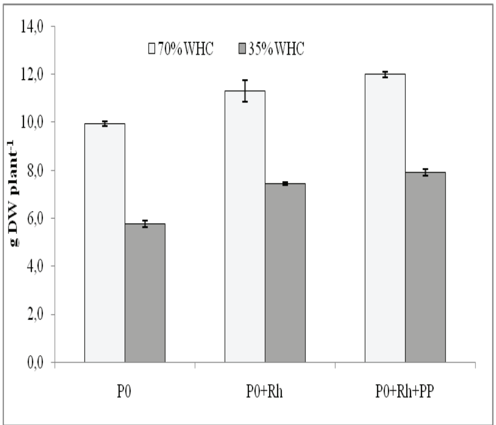

Figure 1. The effect of *Bradyrhizobium japonicum* (Rh) and *Pseudomonas putida* (PP) isolates on dry weight of soybean plants under P insufficiency and moderate drought. Columns are means of four replicates ± SE

Their synergic interaction was observed under both soil moisture levels. The treatments' effect indicated that the combination of *B. japonicum* and *P. putida* increased the dry weight of the plant by 25% over control i.e. P0 under well-irrigated plants. This increment may be due to more absorption of nutrients, especially P due to the increase in root surface area and nutrients available in the soil through rhizobacteria administration (Yadav and Dadarwal, 1997) and the ability of plants to produce phytohormones like gibberellins, auxin, citochinine as well as phosphatases enzymes and other stimulants (Noumavo et al., 2016). Gull et al. (2004) reported that the co-application of phosphate solubilizing bacteria and *Rhizobium* isolates increased P absorption and promoted the growth of pea plants. Soybean plants are sensitive to P deficiency (Israel, 1993). It is documented that phosphorus nutrition plays an important role in crop responses to water stress, but how P fertilization interacts with PGPR, particularly under low moisture of soil, is not elucidated. The application of phosphorus at the rate of 100 mg/kg of soil without inoculation also seemed to be an effective approach for the growth of soybean (Figure 2). However, the integrated application of rhizobacteria and chemical fertilizer promote plant growth and development more significantly as compared with chemical fertilizer alone (P100) under drought conditions.

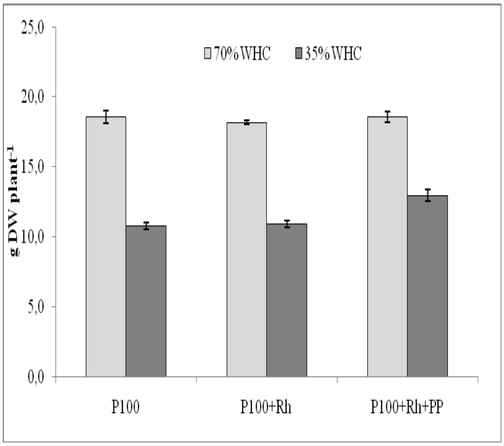

Figure 2. The effect of *Bradyrhizobium japonicum* (Rh) and *Pseudomonas putida* (PP) isolates on dry weight of soybean plants under P fertilization and moderate drought. Columns are means of four replicates ± SE

In general, drought significantly reduced the performance of plant growth irrespective of biofertilzer or P aplication. Therefore, the positive influence of PGPR was also established under drought conditions. In that case, they increased growth by 13.8% in treatment with P insufficiency supply and by 16.2% in the treatment of P supplementation. Probably, the phosphate's solubilization, production of auxin, the fixation of nitrogen and enhanced nutrient uptake are likely responsible for better accumulation of dry matter by soybean (Bashan and Holguin, 1998). The legume's growth correlates with nitrogen atmosphere assimilation and this process is dependent on root nodulation. Hence, nodules provide plants with an alternative nitrogen source due to nitrogen fixation. The enhancement of plant development with biofertilizers and phosphorus led to a better nodule initiation. The supply of phosphorus directly and positively stimulates nodulation in red clover (Hellsten and Huss-Danell, 2000), peas (Jacobsen, 1985) and soybeans (Israel, 1993). There were also other reports suggesting that phosphorus indirectly induced nodulation with a positive effect on the plant growth (Yang, 1995).

Table 1. The effects of *Bradyrhizobium japonicum* (Rh) and *Pseudomonas putida* (PP), phosphorus fertilization and water regime on root nodule number. Mean of four replications ± SE

Treatments	70% WHC	35% WHC
P0	2±0.29	1±0.25
P0+Rh	14±1.04	10±2.55
P0+Rh+PP	29±3.46	13±1.00
P100	3±1.08	2±0.87
P100+Rh	50±1.87	17±1.49
P100+Rh+PP	64±11.88	39±3.57

Experimental data demonstrated that the nodules' growth and development showed significant responses to the application of biofertilizers and phosphorus fertilization, but the response was affected by the water supply level (Table 1). On average, drought decreased the nodule number by 40-123% irrespective of P or PGPR treatments. The significant increase in nodule number under normal water supply both in unfertilized and fertilized soils following inoculation indicated that inoculation with *B. japonicum* is an essential practice for maximum nodulation that would certainly affect the N_2 fixation and N assimilation by soybean plants. So, it was established that combined utilization of these strains significantly increased the number of nodules on soybean roots over the control. Few nodules in uninoculated plants indicated that the indigenous *bradyrhizobia* population was low in the soil (Table 1). Experimental results showed a greater proportion of nodules developed on the root of the soybean after inoculation and phosphorus fertilization.

Drought stress caused a significant reduction in the number of nodules as compared to well-watered plants. In addition, P deficiency essentially diminished the number of nodules, especially under low soil moisture level. The administration of *P. putida* in the soil increased the number of nodules of plants inoculated with *Bradyrhizobium japonicum* (Table 1). It was observed that their combined utilization doubled the number of nodules in plants grown with low P supply under normal water conditions.

The beneficial effect of *P. putida* was established in plants with P supplementation and this parameter increased by 28% at good irrigation levels of the plants and two times more under moderate drought conditions in comparison with the reference uninoculated treatment. The presented results on improved nodulation of soybean induced by *Pseudomonas* confirm the findings by other authors for faba beans (Grimes and Mount, 1984) and lentils (Verma et al., 2013). Therefore, we concluded that the symbiotic system had a positive and significant response to biofertilizers applied separately or in combination irrespective P supply. Our experimental results are consistent with other research obtained in leguminous species (Kadian et al., 2013).

Many studies have demonstrated that rhizosphere microorganisms have a beneficial impact on crop yields (Verma et al., 2013).

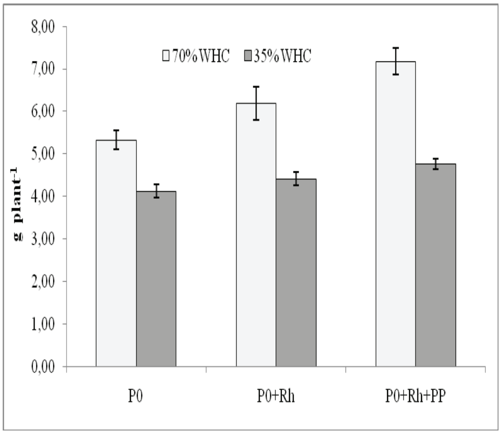

Figure 3. The effect of *Bradyrhizobium japonicum* (Rh) and *Pseudomonas putida* (PP) isolates on grain yield of soybean cultivated on soil with P insufficiency supply under well water and water stress conditions. Columns are means of four replicates ± SE

The use of microorganisms has also created favorable conditions for soybean grain productivity irrespective of soil moisture regime (Figure 3). Low P supply significantly reduced grain production, particularly under temporary drought conditions. Likewise, Tsvetkova and Georgiev (2003) reported that P deficiency treatments in soybean decreased the entire plant dry mass and nodule weight. In our experiment, yield parameters differ significantly between well-watered and drought exposed plants. Data on grain yield (Figure 3) showed that biofertilization as well as phosphorus fertilizer application significantly increased productivity of soybean over the control. The seed treatment with *B. japonicum* increased the yield by 14.1% under normal water conditions. In drought conditions, only a 6.5% increase was observed with respect to control plants without inoculation (Figure 3). We suggest that the water deficit had a direct impact on the leaves' canopy, decreased storage capacity of the source and, subsequently, caused a reduction in seed weight. The percent increase in seed yield due to the inoculation of soybean by two strains used as biofertilizers of unfertilized soil was 19.1% over the control under normal water regime.

Therefore, the combined inoculation produced the maximum result followed by the singular inoculation of *Bradyrhizobium japonicum* compared to the controls. Grain yield was increased by P fertilization and the dry weight of shoots and pods per plant had a significant positive association with grain yield. The integrated application of microorganisms and chemical fertilizer increased the productivity by 19.1% in normal water conditions, while under drought the increase was more modest, around 13.2%, in comparison to the plants without microoganisms administration. Uninoculated plants had the lowest seed yield, probably, because the native rhizobium was ineffective and did not fix much N_2 to increase the growth and seed yield. Egamberdiyeva et al. (2004) reported that the yield of soybean was 48% higher for inoculated than for uninoculated plants grown on calcareous soil. The results of our experiments with *Bradyrhizobium* spp. strains clearly demonstrated that rhizobium inoculation with soil applied with *P. putida* significantly increased soybean seed yield. The drought stress had an undesirable impact on growth, yield and nodulation in all treatments in comparison to well-watered plants. Dashti et al. (1998) revealed that plant co-inoculation of PGPR with *Bradyrhizobium* has been reported to increase legume nodulation and plant tolerance even at low soil temperatures. Similar effects of inoculation with plant growth promoting rhizobacteria and *Sinorhizobium fredii* was observed by Guarcia et al. (2004).

According to the obtained results, we conclude that these microorganisms could provide a better productivity of soybean under phosphorus insufficiency and moderate drought conditions. The significant response of the two strains indicated the synergistic effect of the interaction.

CONCLUSIONS

The results of a greenhouse pot experiment indicate that the soil insufficiency of phosphates in association with drought considerably decreases the growth and productivity of soybean. The seed inoculation with *B. japonicum* strain in combination with soil inoculation with *P. putida* strain significantly increases the plant growth and yield of soybean grain under insufficient phosphorus and moderate drought conditions.

Integrated application of *Bradyrhizobium japonicum* and *Pseudomonas putida* promote nodulation of soybean and increased drought tolerance of soybean.

Inoculation with rhizobium strains alone show less influence in seed yield over the control but the use of both strains *B. japonicum* and *P. putida* increased seed yield significantly.

Hence, certain co-operative microbial species could be used as a low-input biotechnology that provides the stable production of crops and forms the basis for a strategy to develop sustainable and environmental agriculture.

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