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RHIZOBACTERIA EFFECTS ON PHOTOSYNTHETIC PIGMENTS AND NITROGEN CONTENTS OF SOYBEAN PLANTS CULTIVATED UNDER LOW PHOSPHORUS AND WATER LIMITED CONDITIONS

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Abstract

The application of plant growth promoting rhizobacteria (PGPR) is a sustainable approach to improve crops physiological processes and to overcome abiotic stresses. The effects of rhizobacteria Bradyrhizobium japonicum applied alone or in combination with Pseudomonas putida were examined on photosynthetic pigments contents and leaf nitrogen status in soybean plants subjected concomitantly to low phosphorus (P) and moderate drought conditions. The lowest content of pigments was revealed in plants subjected to both abiotic factors. The results indicated that the application of B. japonicum and P. putida significantly improved nitrogen and photosynthetic pigments status in leaves, particularly chlorophyll a and b under temporary drought compared to uninoculated ones. The application of rhizobacteria B. japonicum alone or together with P. putida significantly increased carotenoids concentrations in plants regardless of soil moisture levels. In conclusion, the detrimental effects generated by low P and drought on photosynthetic pigments status and nitrogen nutrition of soybean were partially alleviated by the use of rhizobacteria.

Key words: *chlorophyll, drought, Glycine max L., nitrogen, rhizobacteria.*

INTRODUCTION

The various environmental factors like low soil fertility and drought stress have detrimental impact on plant growth and productivity (Gouda et al., 2018; Cura et al., 2017). Abiotic stresses, provoked by these factors, can hamper a range of physiological processes, particularly photosynthesis and cause damage to plants. The chlorophyll status in plants is an important factor in crop production because it affects the amount of radiation intercepted and, therefore, plant growth (Ashraf & Harris, 2013). Chlorophyll loss is associated with environmental stress, and the variation in total pigments contents may be an estimative indicator of stress in plants (Ashraf & Harris, 2013; Netto et al., 2005). Lisar et al. (2012) found that water stress reduces chlorophyll synthesis, while carotenoids are less affected.

Plant growth-promoting bacteria (PGPB) are reported to influence the growth, yield, photosynthetic activity and nutrient uptake by several mechanisms (Delshadi et al., 2017; Egamberdieva et al., 2017a; 2017b). Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral and nitrogen assimilation and in turn the photosynthetic activity is enhanced. One of the possible mechanisms to increase chlorophylls contents in plants could be associated with improvement of nitrogen status in crops. However, little is known about PGPR impact on nitrogen (N) nutrition of crops when the low P availability is coupled with water deficit in the soil. Reduced N supply is known to reduce the translocation of nitrates, phosphates, calcium, magnesium and amino acids in plants. These physiological disturbances significantly affect the photosynthetic pigment contents in plants. Soybean (*Glycine max*. L.) is a major legume crop and an important source of protein, vegetable oil in many countries; however, its production is restricted by low P availability and drought (Graham & Vance, 2003). To date, little is known regarding the effect of PGPR on the changes in foliar pigments of soybean in relation to P supply and soil moisture levels. Even though *Bradyrhizobium japonicum* and *Pseudomonas putida* have been studied as single isolate affecting leaf chlorophyll status in legumes (Israr et al., 2016; Kang et al., 2014;

Egamberdieva et al., 2017c), little has been done on their possible interactions under water and P limited environments. The aim of this investigation was to evaluate the effect of rhizobacteria *Bradyrhizobium japonicum* applied alone or in combination with *Pseudomonas putida* on the chlorophyll (*a* and *b*), carotenoids and nitrogen content in leaves of *Glycine max* L. under low P fertility and moderate drought conditions.

MATERIALS AND METHODS

The soil used for the experiment was chernoziom carbonated with low P availability (18 mg P/kg soil). In a controlled pot culture experiment, a set of soybean (cv. Horboveanca) plants were inoculated with rhizobacteria *B. japonicum* (Rh) alone and another set of plants were inoculated with *B. japonicum* in combination with soil applied *P. putida* (PP). In addition, it was a variant with P fertilization at the rate 100 mg P/kg soil. Uninoculated plants served as control treatment. Pots were arranged in a randomized complete block design with four replications. All plants were well-watered till flowering stage. After that, a set of plants were watered at 70% WHC (water holding capacity) as normal soil moisture and the other set of plants was subjected to low water conditions (35% WHC) for 12 days. After that period leaves samples were collected to determine photosynthetic pigments contents. To determine the chlorophyll content, leaf samples were extracted with acetone (80%). The absorbance of the supernatant was recorded at 645 and 663 nm against the solvent (acetone) (Arnon, 1949). The carotenoids concentration was measured at 452 nm. The total nitrogen content was determined by Kjeldahl method. The obtained data were given as the mean with standard error values by using Statistic 7 program, and the differences in the means were determined by the least significant difference (LSD) (P=0.05) test.

RESULTS AND DISCUSSIONS

Morphological, nutritional and physiological changes induced in plants colonized with PGPR contribute to their enhanced growth and resistance to abiotic stresses**.** Variations in chlorophyll patterns in leaves are indicators of senescence, stress or damage to the photosynthetic apparatus and affect the normal growth of plants. In this study, we investigated whether the application of beneficial bacteria *B. japonicum* alone or in combination with *P. putida* have potential to improve chlorophylls status in soybean cultivated under limited P supply and temporary drought. The effects of these bacterial strains on the chlorophylls contents in plants are shown in Figure 1.

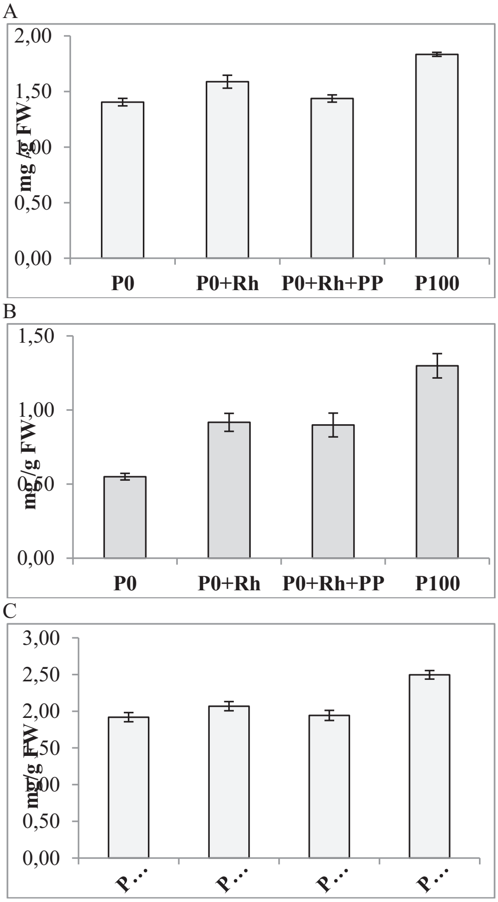

Figure 1. The effect of *Bradyrhizobium japonicum* (Rh) applied alone or in combination with *Pseudomonas putida* (PP) on (A) chlorophyll *a*, (B) chlorophyll *b* and (C) total chlorophylls content in leaves under low P and normal soil moisture. Values exhibit means ± SE

Experimental results revealed the lowest content of pigments in plants cultivated under low P availability and subjected to moderate drought. The decrease in chlorophyll (*a* and *b*) contents in drought-affected soybean plants might be attributed to the possible oxidation of chlorophyll and other chloroplast pigments coupled with instability of the pigment protein complex under abiotic stress. Similar trends have been reported by Curá et al. (2017) who revealed that drought stress significantly decreased the total chlorophyll content in leaves of maize plants.

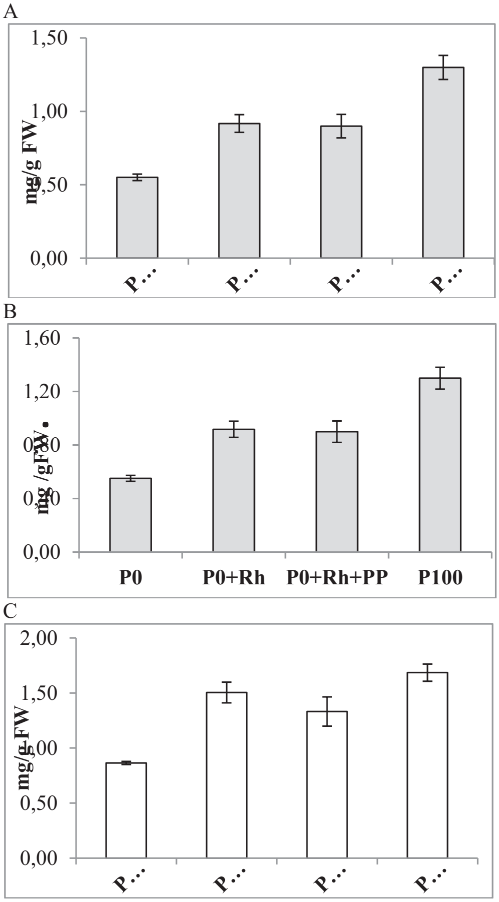

Figure 2. The effect of *Bradyrhizobium japonicum* (Rh) applied alone or in combination with *Pseudomonas putida* (PP) on (A) chlorophyll *a*, (B) chlorophyll *b* and (C) total chlorophylls content in leaves under low P and drought. Columns are means ± SE

The experimental data of this study have shown that the application of rhizobacteria *B. japonicum* improved the photosynthetic pigments contents in soybean plants under normal soil moisture as well as under water stress (Figures 1 and 2).

The content of chlorophyll *a* and chlorophyll *b* was increased by 13.6% and 76.9% due to *B. japonicum* inoculation in plants not subjected to water deficit. Plants inoculated with the combination of *B. japonicum* and *P. putida* did not increase photosynthetic pigments further compared to the inoculated plants with *B. japonicum* alone. The highest concentration of pigments was registered in treatment with P fertilization irrespective of soil moisture levels. Sufficient supply of P contributes to uptake and assimilation of nutrients especially of Mg as was reported by Vafadar et al. (2014). In addition, higher chlorophyll content in response to P supplementation might account for synthesis of more carbohydrates in fertilized plants. Investigation data revealed that the application of rhizobacteria also improved the chlorophyll *a* in soybean plants under water stress. Likewise, the chlorophyll *b* content was increased significantly in all the PGPR strain treatments. Indeed, the application of *B. japonicum* alone increased chlorophyll *a* and *b* by 67.2% and 90.3% under temporary drought compared to uninoculated plants. An increase of chlorophyll content in plants contributes to higher photosynthetic activity and assimilates production, which could be supported the plant growth improvement under unfavorable P supply and drought environment. Similar results have been reported by Mia et al. (2010), who treated banana plants with chemical fertilizers and PGPR and observed positive effects on chlorophyll contents and Ca, Mg, K uptake by plants. Kang et al. (2014) also demonstrated a stimulatory effect of *P. putida* strain on chlorophylls accumulation in soybean under salinity stress. In this study, the stimulatory effect of rhizobacteria on total pigments contents was registered in plants grown under water deficit environment. However, under insufficient supply of water the major increases were observed in inoculated plants compared to uninoculated ones. In general, plants subjected to drought irrespective of treatments had low concentration of chlorophylls compared to well-watered ones. Manivannan et al. (2008) showed that under drought stress the amount of chlorophyll and carotenoids was reduced in *Helianthus annuus*. It seems that the reason for this decrease is due to the increased destruction and/or production of pigments as consequences of oxidative stress generated by stress of abiotic factors. Likewise, the reduced chlorophyll contents of the plant under unfavorable environments might be somewhat due to limited N supply in plant tissues and changes in enzymes activity such as nitrate reductase (Delshadi et al., 2017). However, the application of tested PGPR strains attenuates adverse effects of drought on chlorophyll. Esitken et al. (2010) reported that the use of growth promoting bacteria of *Pseudomonas* increased the amount of Fe and Mg in strawberry, hence, created favorable physiological conditions for pigment synthesis. We also can suppose that tested bacterial strains alleviated iron and magnesium nutrition of soybean plants. Our results of enhanced chlorophyll contents due to PGPR application and subsequent allaying of drought induced negative effects support the results for fenugreek (Sharghi et al., 2018). Thus, the enhanced chlorophylls status in plants was because rhizobacteria treated plants maintain higher contents of magnesium and N resulting in greater physiological activity of chloroplasts (Egamberdieva et al., 2017a). Carotenoids play an important role in the process of reactive oxygen species scavenging, in the stability of photosynthetic machinery activity; participate in energy dissipation hence contributing to the reduction of adverse effects generated by unfavorable environmental conditions (Ashraf & Harris, 2013). In our experiment, the carotenoids contents in plants were detrimentally affected by drought and P deficiency. The decrease in carotenoids status due to drought and low soil fertility of P could be related to limited nutrients availability in leaves tissues. In addition, it is known that under scarce conditions of P and water deficit the uptake of Mg is restricted. The application of *B. japonicum* alone increased this physiological parameter in plants (Figure 3). This elicitor influence of rhizobacteria was observed irrespective of soil moisture levels. It is necessary to note that the leaves biomass in treatment with both strains was significantly higher than in treatment with *B. japonicum* alone (data not shown). The total carotinoids content increased by 63.2% in plants not subjected to water limited conditions and by 54.4% in soybean plants under drought due to inoculation only with N-fixer rhizobacteria (Figure 3A). However, the combined application of two bacteria strains did not increase this parameter under drought conditions compared to treatment of *B. japonicum* alone (Figure 3B). Generally, the carotenoids accumulation decreased in plants without rhizobacteria treatment regardless of soil moisture level. One of the mechanisms induced by plant growth promoting bacteria is improving plant nutrition, especially with nitrogen.

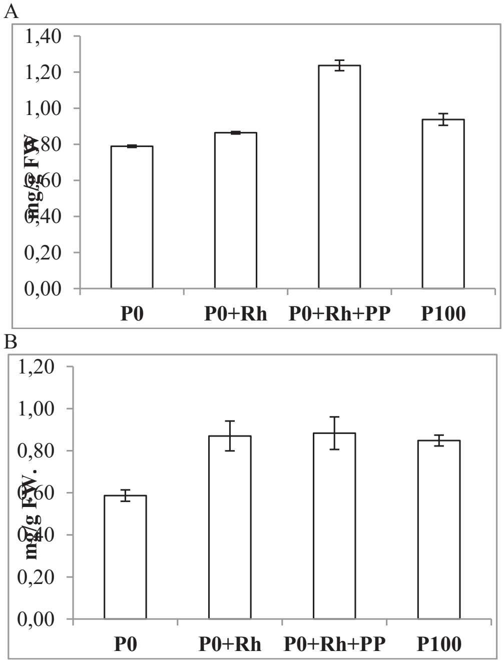

Figure 3. The effect of *Bradyrhizobium japonicum* (Rh) applied alone or in combination with *Pseudomonas putida* (PP) on carotinoids content in leaves under (A) normal soil moisture and (B) drought. Columns are means ± SE

According to literature data there is a strong correlation between photosynthetic pigments synthesis and nitrogen status of plants. For example, Dawwam et al. (2013) reported that the stimulation of pigment synthesis in potato plants by PGPR might be due to the changes in N, P and K uptake. The study's results found out that the *B. japonicum* inoculation treatment in combination with *P. putida* was the most effective treatment for increasing the foliar N concentration in plants under temporary drought. We'd like to mention that the co-inoculation effect of *Bradyrhizobium* and *P. putida* enhanced nodulation and root growth compared to the control plants (data not shown), which in turn have beneficial impact on nitrogen assimilation by soybean. Exposure of soybean to water stress reduced accumulation of essential mineral elements like nitrogen and phosphorous resulting in decrease of growth. Altogether, increased N content in inoculated plants has positive influence on the synthesis of chlorophyll pigments resulting in enhanced synthesis of photoassimilates and hence leads to improvement of resistance to abiotic factors.

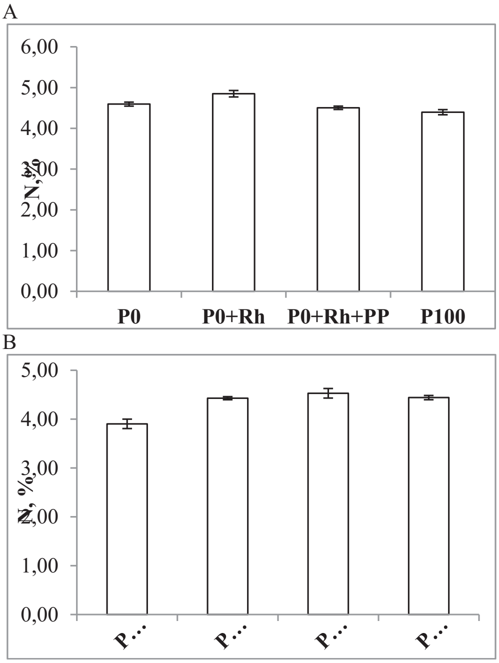

Figure 4. The effect of *Bradyrhizobium japonicum* (Rh) and *Pseudomonas putida* (PP) isolates on N content in leaves under normal soil moisture (A) and moderate drought (B). Columns are means ± SE.

Under normal water soil conditions rhizobacteria did not significantly change the nitrogen concentration in leaves tissues of soybean plants (Figure 4A). The administration of bacterial strains increased the nitrogen status in leaves of plants grown under unfavorable soil moisture conditions. The differences in nutrient contents between uninoculated soybeans and those inoculated together with *B. japonicum* and *P. putida* were significant, the nitrogen content being improved by 25% (Figure 4B). Perhaps, these rhizobacteria stimulated root growth and its physiological activity. Experimental results also suggested an increase in nodule number in soybean upon bacterial inoculation under drought (data not shown). Our experimental results agrees with the experiment by Egamberdieva et al. (2017c), where lupine inoculated with the *Bradyrhizobium* strain and with the HTC-BR inoculants contained 11% and 33% higher nitrogen contents under irrigation, and even 21% and 39% under drought relative to uninoculated plants, respectively. In uninoculated plants, the contents of total nitrogen were similar for plant tissues under drought as well as for those of non-stressed ones. According to experimental results regarding N status we can make a conclusion that combined applications of both rhizobacteria species had better impact on nitrogen status in plants compared with the inoculation with *B. japonicum* alone under temporary drought. Egamberdieva et al. (2017b) demonstrated that both inoculation treatments, either *B. japonicum* USDA 110 alone or combined with *P. putida* TSAU1, enhanced nitrogen contents in salt-stressed soybean tissues. Likewise, Israr et al. (2016) suggested that the application of *P. putida* strain not only enhances P uptake, but also improves the N and K uptake by chickpea plants grown on high pH soils, which can thus reduce the application of chemical fertilizers. In this study, the phosphorus supplementation provides the same status of nitrogen as rhizobacteria application.

CONCLUSIONS

Inoculation of soybean with *Bradyrhizobium japonicum* improved photosynthetic pigments status in plants under normal soil moisture as well as under water deficit and low P fertility of soil. Combined application of *B. japonicum* and *P. putida* had no synergic effect on chlorophylls status of plants regardless of soil moisture level. The combined application increased significantly the nitrogen contents under scarce moisture conditions. Thus, experimental results suggested that PGPR enabled the leaf to maintain high levels of photosynthetic pigments and nitrogen and reduced the adverse effects of water deficit and low P on soybean plants. These findings were obtained in greenhouse conditions; therefore, it would be necessary to carry out further experiments under field conditions which would be quite important research for sustainable crop production under unfavorable environments.

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