EFFECTS OF PHOSPHORUS FERTILIZER AND PLANT GROWTH PROMOTING RHIZOBACTERIA ON THE CHLOROPHYLL AND NITROGEN CONTENT IN SOYBEAN UNDER SUFFICIENT AND LOW WATER SUPPLY

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Abstract

Nitrogen is a crucial element in the production of both leguminous and non-leguminous crops and has pivotal impact on the growth of legumes. The yield of soybean mainly d[1]depends on the accumulation of nitrogen and chlorophyll content in leaves. A pot experiment was conducted to determine the effects of phosphorus (P) and plant growth-promoting rhizobacteria (PGPR) treatments, applied in combination with *Pseudomonas fluorescence* and *Azotobacter chroococcum* on the chlorophyll and nitrogen content in soybean (*Glycine max. (L) Merr*). P and PGPR were applied in the soil before sowing. Soybean plants (cv Zodiac) were grown on soil-sand mixture with P deficiency at two water regimes - 70% water holding capacity of soil (WHC) as sufficient supply and 35% WHC as low water supply. The results revealed an overall increase in chlorophyll *a* and total chlorophyll content in the PGPR treated plants, compared to control (unfertilized) samples under normal soil moisture conditions. With regard to P supply, chlorophyll content was uniform in all treatments and there were no significant changes of chlorophyll concentration in normal soil water regime. However, phosphorus supply decreased chlorophyll concentration under water stress conditions. Phosphorus fertilization and PGPR application increased significantly the nitrogen concentration in leaves of cv. Zodiac under sufficient water supply. Under low water supply the P application increased the concentration of nitrogen in roots in comparison to control plants. Hence, the results suggest that the utilization of rhizobacteria and P supplemental nutrition have the potential to enhance the chlorophyll and nitrogen content of soybean in normal water regime of soil.

Key words: chlorophyll, nitrogen, phosphorus, rhizobacteria, soybean

Soybean, as a major grain and forage crop, is significantly affected by P deficiency and drought. The yield of soybean mainly depends on the accumulation of nitrogen and chlorophyll content in leaves. Nitrogen is a building block of proteins and amino acids which are required for the cellular synthesis of enzymes, chlorophyll, DNA and RNA, and therefore, is important in plant productivity (Wood C.W., Torbert H.A., Weaver D.B, 2003). Legumes in comparison with cereals consume a large quantity of nitrogen (Vance C.P. *et al.* 2003). Nitrogen supply has a significant effect on leaf growth because it increases the leaf area of plants and consequently it influences the photosynthesis activity. There is large experimental evidence that nitrogen assimilation and chlorophyll contents in crops depend on P supply. It is well documented that a low level of P in soil and an insufficient water regime are major constraints to crop growth and production in many agricultural regions, in particular in the republic of Moldova (Andires S., 2007). The ability of soil microorganisms to convert insoluble forms of phosphorus into a soluble form is an important trait in plant growth-promoting bacteria for increasing the photosynthetic activity of plants (Vessey K.J., 2003). Different species of *pseudomonas and azotobacter* could stimulate plant growth via different mechanisms such as antibiotics synthesis, plant hormone production, increasing P absorbance by plant (Abdul-Jaleel J. *et al.,* 2007).

Therefore, a study was conducted with the aim of assessing the effects of PGPR application *pseudomonas fluorescence and azotobacter chroococcum* and phosphorus (P) supplementation on nitrogen and chlorophyll content in soybean plants in relation to water supply.

MATERIALS AND METHODS

A pot experiment was conducted under a controlled soil moisture regime. The soybean (*Glycine max. L. Merr*) cultivar Zodiac was used in this investigation. The soil for the pot experiment came from the carbonated chernoziom soil experimental field (0 ~ 30 cm topsoil was used). It was

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characterized with pH 8 and low available phosphate (1,8 mg/100 g soil). The soil was sieved and then mixed with sand at a ratio 3:1. 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 fluorescence* and *Azotobacter chroococcum* was applied by spraying the soil with the bacteria suspension and then thoroughly homogenize. The pots were separated into two sets, each having four replicates for all treatments. Before the sowing, the soybean seeds were treated with bacteria *bradyrhizobium japonicun*. The two water treatments were at 70% water holding capacity (WHC) as control and at 35% WHC as insufficient water supply. The water deficit was initiated at the flowering stage for 12 days. 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 chlorophyll and carotinoids estimation in leaves were done following the method of Arnon (Arnon D.I., 1949). Total nitrogen in the ground material of plants was estimated by Kjeldhal digestion, distillation and titration method (Mineev V., 1989). The data was analyzed statistically by factorial ANOVA using Statistics software version 8.1 techniques and comparison among mean values of treatments was made by the least significant difference.

RESULTS AND DISCUSSION

Results indicated that different treatments showed different leaf chlorophyll contents in soybean in both water soil regimes (*table 1 and 2*). The total leaf chlorophyll concentration significantly increased with rhizobacteria application compared to uninoculated pots. Rhizobacteria (*Pseudomonas fluorescence and Azotobacter chroococcum*) application in the carbonated chernoziom showed increase in leaf chlorophyll *a* and total chlorophyll content by 9% and 11% respectively to the control (unfertilized). Similarly, Anjum M.A. and coworkers (Anjum M.A. *et al.* 2007) reported that beneficial rhizobia bacteria may influence the growth of cotton plants by increasing the chlorophyll content in leaves.

Table 1

Effects of phosphorus fertilization and microorganisms (MO) application on soybean chlorophyll content (mg/g) under normal soil water regime (70% WHC). Values are means (*n* = 3)

Treatments	*a*	*b*	*a+b*	carotinoids
P0	2.30	1.00	3.32	0.99
P0+MO	2.51	0.98	3.56	0.82
P20	2.24	0.92	3.04	0.90
P100	2.49	1.08	3.63	0.99

Supplementation of P at 100 mg/kg resulted in greater values of total chlorophyll and chlorophyll *a* content compared to unfertilized plants. In all treatments there were no significant changes of chlorophyll *b* concentration under a sufficient water soil regime. Regarding the carotinoids concentration we could mention that the use of rhizobacteria decreased their concentration in leaves, while P supplementation did not change this parameter.

Table 2

Effects of phosphorus fertilization and MO application on soybean chlorophyll content (mg/g) under insufficient water supply (35% WHC). Values are means (*n* = 3)

Treatments	*a*	*b*	*a+b*	carotinoids
P0	2.41	1.17	3.62	1.02
P0+MO	2.28	0.92	3.24	0.91
P20	2.13	1.10	3.17	1.05
P100	2.11	0.99	3.25	0.93

In drought conditions the leaf green pigments concentration decreased under P fertilization. This trend was established irrespective of the rate of chemical fertilizer (*table 2*).

With regard to P supply, chlorophyll *b* content was uniform in all treatments. Also, the concentration of carotinoids did not change significantly under treatment with PGPR as well as by P fertilization. Thus, from this study we can conclude that P supplementation at both levels did not show significant effect on the chlorophyll content of the soybean under a suboptimal water supply. Our previous study demonstrated the positive impact of PGPR application on soybean growth due to the improvement of phosphorus nutrition (Rotaru V., 2013). In this study we examined the changes of nitrogen concentration in soybean parts in relation to mineral P supplementation and biofertilizers application. It is well documented that the nitrogen content in plant tissues is closely related to the synthesis of chlorophyll (Salisbury F.B., Ross C.W., 1992).

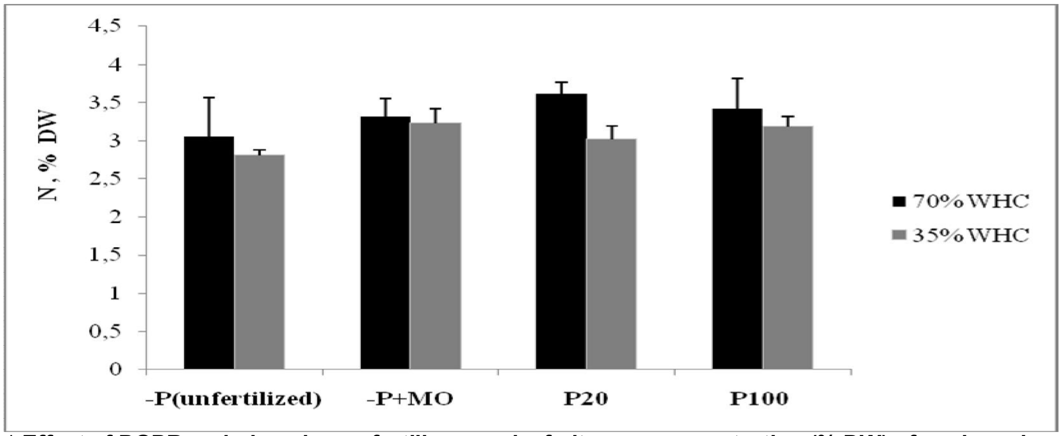

Figure 1 **Effect of PGPR and phosphorus fertilizers on leaf nitrogen concentration (% DW) of soybean in relation to water supply. Data are means of four replicates. Bars represent standard errors.**

The effect of P-supply and rhizobacteria administration on the nitrogen content in different soybean organs (%, DW) is illustrated in figure 1, 2 and 3. Different treatments had different impact on the leaf nitrogen content of soybean. Nitrogen content was higher in leaves than in roots and stems regardless of treatments. Our experimental results revealed the beneficial effect of the rhizobacteria application on nitrogen concentration in soybean irrespective of soil water regime. Similar to our work, other studies have also shown that PGPR increased the nitrogen content in the legume plant, ending up with improved plant growth (Garcia L. *et al*., 2004).

In cv. Zodiac, a higher increase (14%) in leaf nitrogen was recorded in P supplementation treatment as to untreated control. Therefore, total nitrogen content in plants can influence the outcome of photosynthesis via the photosynthetic enzymes and chlorophyll formation. However, single application of P showed decrease of nitrogen concentration in stems (*figure 2*). The examination of plants subjected to temporarily insufficient water supply revealed that the P supplementation of soybean plants as well as MO application increased the nitrogen concentration in the leaves of Zodiac. Also, the fertilization with P improved the N status in roots, increasing this parameter by 17%.

Under optimal P-supply, N-content was lower in stems (*figure 2*) of *Glycine max* than in control plants. Under conditions of deficient P, a significant reduction in N-content in roots was observed in low water regime in comparison to sufficient P nutrition (*figure 3*). This influence was less evident under normal water supply. We can conclude that the leaves were more negatively affected by P-deficiency in both soil water regimes compared to the roots.

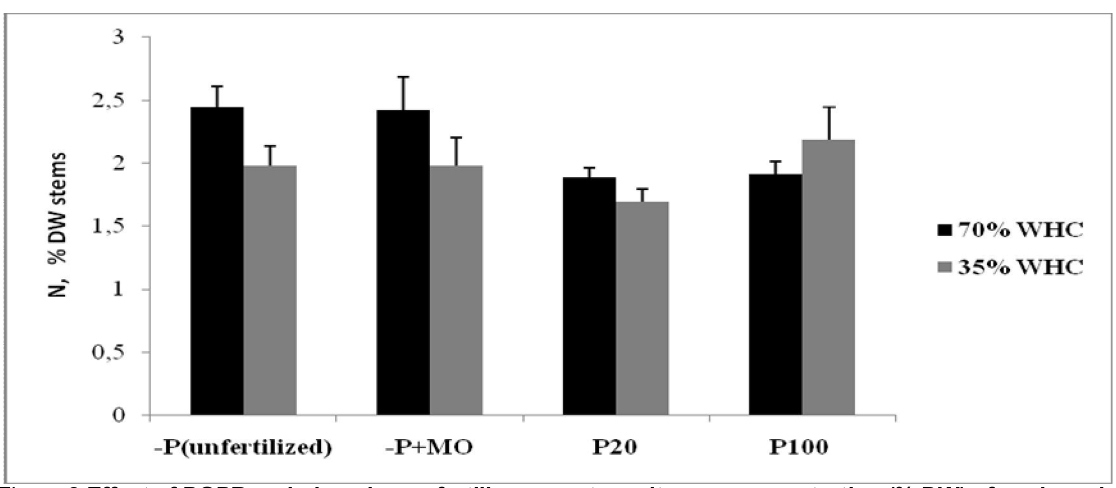

Figure 2 **Effect of PGPR and phosphorus fertilizers on stem nitrogen concentration (% DW) of soybean in relation to water supply. Data are means of four replicates. Bars represent standard errors**

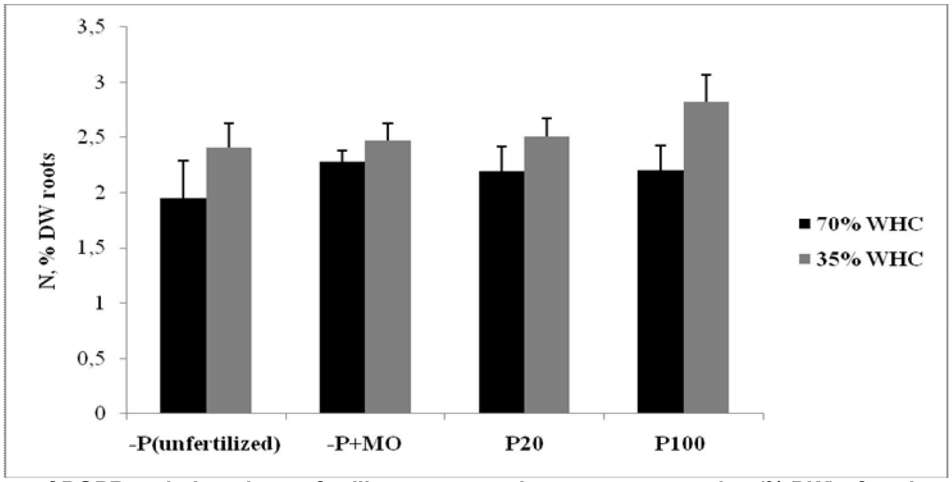

Figure 3 **Effect of PGPR and phosphorus fertilizers on root nitrogen concentration (% DW) of soybean in relation to water supply. Data are means of four replicates. Bars represent standard errors**

We suppose that the higher N uptake due to P supply and biofertilizers is ascribed due to (a) the increase in root mass because P nutrition resulted in the absorption of a higher concentration of mineral nutrients from soil including N and (b) the significant increase in number and mass of root nodules due to P and PGPR increased nitrogen fixation that lead to an increase in N uptake. Therefore, the experimental results demonstrated that the use of PGPR and the supplementation of P displayed a potential to increase the contents of chlorophyll and nitrogen in cultivar Zodiac, in particular under normal water supply.

CONCLUSIONS

Our results showed the comparative effect of PGPR and P application on the leaf chlorophyll content and nitrogen in vegetative parts of soybean in relation to water supply. The response of chlorophyll and nitrogen contents in soybean to PGPR application depends on the soil water regime. The chlorophyll and nitrogen content with PGPR administration increased under normal soil water regime over the control treatment. However, a field evaluation is necessary to confirm their beneficial impact on green pigment accumulation and nitrogen assimilation of soybean in order to assess the practical utility of these bacteria.

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