CHANGES IN NUTRIENT STATUS OF SOYBEAN IN RELATION TO *BRADYRHIZOBIUM JAPONICUM* AND SALYCILIC ACID APPLICATION UNDER LOW PHOSPHORUS AND WATER SUPPLY

MODIFICĂRI ÎN STATUSUL NUTRITIV LA SOIA ÎN DEPENDENȚĂ DE APLICAREA *BRADYRHIZOBIUM JAPONICUM* ȘI ACIDUL SALICYLIC ÎN CONDIȚII INSUFICIENTE DE FOSFOR ȘI UMIDITATE

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Abstract.

Phosphorus (P) deficiency of soil and drought are major environmental constraints, which alter key physiological constituents and functions in plants. The study was carried out to determine the influence of rhizobacteria Bradyrhizobium japonicum and salicylic acid (SA) on particularities of soybean mineral nutrition under low P supply and drought conditions. Plants were treated with two regimes of irrigation water: normal soil moisture, 70% of water holding soil capacity (WHC) and 35% of WHC as drought. The bacterial suspension of Bradyrhizobium japonicum was applied as seeds treatment and SA by foliage treatment (0,5mM). Plants cultivated under P insufficiency and drought exhibited lower physiological parameters. Experimental data demonstrated that integrated application of nitrogen-fixer bacteria and SA better improved phosphorus and potassium contents in leaves.

Key words: *rhizobacteria, acid salicylic, soybean, phosphorus, nutrients*

Rezumat.

Deficitul de fosfor din sol și seceta sunt impedimente majore ce au repercursiuni negative asupra starii fiziologice a plantelor. S-a organizat un studiu pentru a determina influența aplicării rizobacteriilor Bradyrhizobium japonicum și acidului salicilic (AS) asupra particularității nutriției minerale a plantelor de soia cultivate în condiții de deficit de fosfor și secetă. Plantele au fost supuse la două regimuri de umiditate a solului: umiditate optimă, 70% din capacitatea totală de apă a solului (CTAS) și deficit de umiditate, 35% CTAS. Tulpinile de B. japonicum au fost administrate la tratarea semințelor, iar AS în concentrație de 0,5mM prin tratarea plantelor în perioada de vegetație. Indicii fiziologici la plantele cultivate la insuficiența de fosfor și secetă au înregistrat valorile cele mai mici. Rezultatele experimentale au demonstrat că aplicarea integrată a tulpinilor fixatoare de azot și AS au îmbunătățit nutriția plantelor cu fosfor și potasiu.

Cuvinte cheie: *acid salicilic, rizobacterii, soia, fosfor, nutrienti*

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INTRODUCTION

Soybean is the main leguminous crop and is cultivated for high yields of protein and vegetable oils needed for human food and animal feed. In the Republic of Moldova soybean yields are low in particular in drought conditions. The major impediment to fully realizing productivity is poor soil fertilization, particularly in carbonate chernozem with low phosphate levels and insufficient soil moisture [Andrieș, 2011]. An insufficient supply of mineral elements and water to agricultural plants impairs the physiological functioning of the photosynthetic apparatus, including photosynthetic pigment synthesis, and reduces the efficiency of nutrient and moisture utilization from the soil. Improving the microbiome has been adopted as an option to stimulate plant physiological functions under adverse conditions [Singh et. al., 2023]. It is known that the B. japonicum plays an important role in enhancing plant growth and enhancing plant resistance under unfavorable conditions [Egamberdieva *et al.*, 2017]. There are many reports demonstrating that *Bradyrhizobium japonicum* strains interact with soybean plants setting a symbiotic system that benefits plant growth and nutrition [Kothari *et al.*, 1990]. Another strategy to mitigate the effects of abiotic stress factors on plants is the use of phenolic growth regulators [Vurukonda *et. al.*, 2016]. Among them, acid salicylic (AS) is a phytohormone with regulatory action in physiological processes and plays an important role in triggering protective mechanisms against biotic and abiotic stresses [Kabiri *et al.*, 2014; Urban *et al.*, 2022; Szalai *et al.*, 2000]. Under water deficit, the ameliorating effect of salicylic acid is determined by the trigger activities of antioxidant enzymes [Ștefarța *et al.*, 2014] as well as by the accumulation of osmolytes that provide a more effective protective system for plants. Although it has been documented that both rhizobacteria and AS show a stimulatory effect on plants, the effect of their combined application on some physiological characteristics of plants grown under unfavorable environmental conditions is not fully elucidated. To date, however, little research has been conducted on the effects of *B. japonicum* inoculation in combination with foliar AS application on changes in nitrogen, phosphorus, and potassium content in soybean plants subjected to drought and low phosphorus availability in the soil. We speculated that the rhizobia and AS may display synergism for plant growth and improvement of nutrient status of plants. The aim of the research was to evaluate the effect of *Bradyrhizobium japonicum* and salicylic acid application on the nutrient status of soybean grown under conditions of low supply of phosphorus and soil moisture.

MATERIAL AND METHOD

To accomplish the purpose of the study a pot experiment was conducted in the vegetation complex of Institute of Genetics, Physiology and Plant Protection, MSU, Republic of Moldova. Plants were grown on carbonate chernozem with low mobile phosphate content. All pots were well watered at 70% water holding capacity (WHC) until drought stress was imposed. The application of rhizobacteria was carried out by

treating soybean seeds (cv Horboveanca) with *Bradyrhizobium japonicum* (Rh) before sowing. Insufficient soil moisture (35% WHC) was installed at the flowering stage for a period of 12 days. A set of plants were twice treated with 0,5mM salicylic acid (SA) solution in the branching and early flowering phases. All variants were set up in eight replicates. The changes in nitrogen, phosphorus, potassium and water contents in leaves were examined as a function of rhizobacteria and AS application. At the end of the drought, the leaves were sampled. The total nitrogen content was determined by Kjeldahl method. The phosphorus content was determined using a spectrophotometer as described by Murphy and Riley (1962). Potassium in leaves was determined by flame photometer [Mineev, 1989]. Plant water status was estimated by determining the relative water content in leaves [Turner, 1981]. Data were statistically analyzed using analysis of variance (ANOVA) by Statistic program 7 and presented as treatment mean ± SE of three replicates.

RESULTS AND DISCUSSIONS

Abiotic factors have a negative impact on plant physiology of soybean. Low soil fertility and insufficiency of soil moisture causes nutritional imbalance in plants. However, little is known about the impact of rhizobacteria *Bradyrhizobium japonicum* and AS application on particularities of plant mineral nutrition under low P and low moisture of soil. Literature data have shown that rhizobacteria have the ability to beneficially influence the nutrient uptake and utilization by crops. Therefore, it was important to examine the impact of the treatments on the nitrogen, phosphorus and potassium contents in leaves of soybean plants. The changes of N concentrations in leaves in relation to rhizobacteria and salicylic acid application are shown in *Figure 1*.

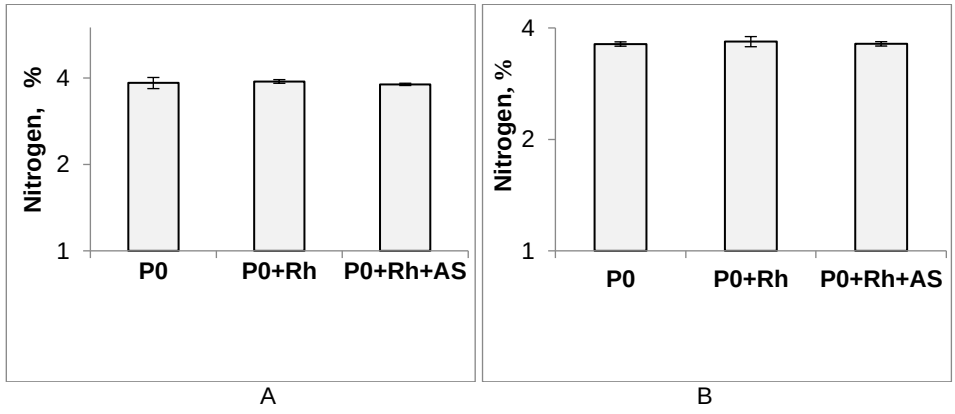

Fig. 1. The influence of *B. japonicum* inoculation alone or in combination with AS treatments on nitrogen content in leaves of soybean grown under normal moisture (A) and low soil moisture (B). Values represent the mean value of 3 replicates ± SE**.**
Note: P0 – low phosphorus; Rh - *Bradyrhizobium japonicum*; AS - acid salicylic.

Nitrogen content in leaves showed that the use of rhizobacteria both separately or in combination with foliar treatment of plants with salicylic acid did not

significantly alter this physiological parameter. Thus, it has been revealed that the application of rhizobacteria to soybean plants did not produce significant differences in the nitrogen content in the leaves of plants grown under optimal moisture of soil compared to control plants (without inoculation). The same effect was found when rhizobacteria were used separately or in combination with AS treatments on leaf nitrogen content under suboptimal (35% WHC) soil moisture conditions (*Fig. 1B*). Adequate phosphorus nutrition has an important function in maintaining the physiological activity of plants at the higher level. According to data of the literature, sufficient phosphorus supply ensures better drought tolerance of crops. Analysis of the data obtained in this experiment from plants grown under optimal moisture conditions (*Fig. 2A*) showed that the use of bacteria in combination with AS treatments contributed to the improvement of phosphorus nutrition compared to the control variant (without rhizobacteria application). In this case, the phosphorus content in leaves increased up to 6.22 mg/g while in the control variant this parameter was 5.57 mg/g. The highest phosphorus content was registered in plants of the variant with integrated use of rhizobacteria and AS. Under water deficit conditions, the lowest percentage of P registered in control plants, which were not inoculated and received an insufficient phosphorus supply (*Fig. 2B*). Also, in soybean plants subjected to temporary drought it was observed that the integrated use of *Bradyrhizobium japonicum* and AS provided better phosphorus nutrition than the application of *B. japonicum* bacteria alone. These results suggest that the improvement in phosphorus nutrition due to the treatments had a beneficial impact on plant growth under conditions of low phosphorus and limited water supply (data not sown).

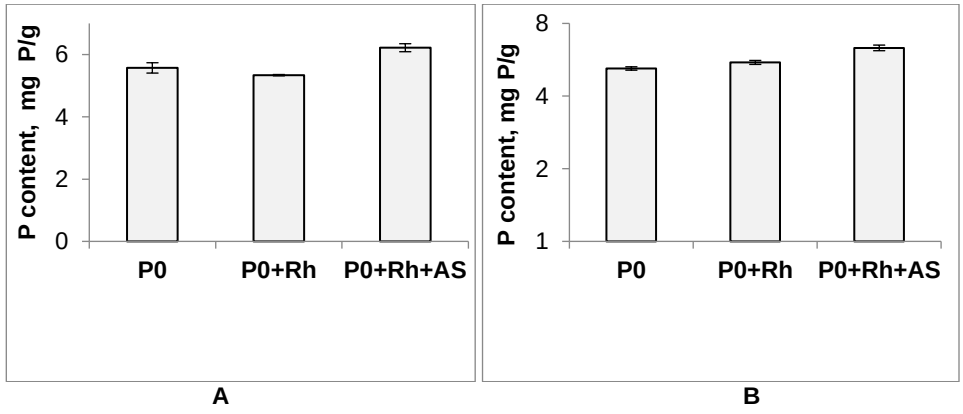

Fig. 2. The influence of *B. japonicum* alone or in combination with AS on phosphorus content in leaves of soybean grown under normal soil moisture (A) and low soil moisture (B). Values represent the mean value of 3 replicates ± SE.

Thus, it was revealed that the integrated application of bacterial strains and salicylic acid increased the phosphorus content in leaves to 6.34 mg/g versus 5.21 mg/g

recorded in the control plants. We believe the higher phosphorus content in leaves, resulting from the combined application of bacterial strains and the growth regulator AS, is due to the development of a more vigorous root system, which ensures more efficient uptake and translocation of phosphorus from the nutrient medium to the aerial parts of the soybean. Other researchers have also demonstrated the stimulatory effect of bacterial strains and salicylic acid on nutrient accumulation in plants. For instance, Ghazijahani and co-workers (2014) reported that foliar treatments with this phytohormone or citric acid altered the dynamics of nutrient acquisition in plants of *Ocimum basilicum* L. After nitrogen and phosphorus, potassium plays an important physiological function both in the growth process and in the osmotic adjustment of plant cells under abiotic stress. A number of external environmental factors control the accumulation of this nutrient in plants. This study investigated whether the application of rhizobacteria *B. japonicum* and AS has the potential to modify the potassium content in leaves of plants. The data presented in *Table 1* show that the application of rhizobacteria separately or in combination with AS weakly affected this leaf parameter in plants grown at optimal humidity of soil (70% WHC).

Table 1

The influence of *Bradyrhizobium japonicum* (Rh) and acid salicylic (AS) on potassium contents in leaves of soybean cultivated under insufficiency of phosphorus and low soil moisture, % average ± standard error

Variant	70% WHC	35% WHC
P0	1.79±0.01	1.91±0.02
P0+Rh	1.67±0.03	2.06±0.02
P0+Rh+AS	1.64±0.01	2.05±0.04

Examination of the data obtained from plants subjected to water deficit of soil revealed that the combined application of *B. japonicum* and AS increased potassium content by 7.3% compared to the control variant where this index was 1.91%. One of the reasons for the positive impact of treatments can be explained by the fact that rhizobacteria producers phytohormones, in particular AIA (3-acid indolyl acetic), gibberellins that stimulate the development of the root system, thus increasing root surface area which consequently increases the rate of nutrient uptake from the soil [Naseem *et. al.*, 2018]. It has been stipulated that improving potassium nutrition have a beneficial impact on water status as well as on the assimilation of nitrate, phosphate and other nutrients, which also contributes to strengthening the physiological functions of plants and increases plant tolerance under adverse environmental conditions [Khan *et. al*., 2015].

Relative water content (RWC) in leaves is an important physiological marker in the assessment of water balance in plants. According to literature data, the use of both rhizobacteria species and phenolic growth regulators [Naseem *et. al.*, 2018; Urban *et. al.*, 2022] modifies to some extent the drought tolerance of plants,

providing better conditions for water uptake and nutrients utilization efficiency. Experimental results of the study revealed changes of relative water content of soybean plants in relation to application of rhizobacteria and salicylic acid treatments (*Fig. 3 and 4*).

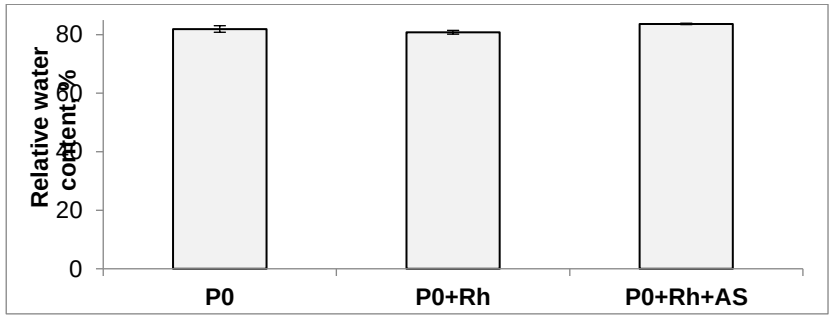

Fig. 3. The influence of *B. japonicum* and AS on relative water content in leaves in soybean grown under low P and normal soil moisture (70% WHC)

The combined use of rhizobacteria *Bradyrhizobium japonicum* and salicylic acid improved leaf water status in plants grown under phosphorus deficiency, especially of soybean grown under normal moisture conditions. Likewise, their beneficial effect was also observed in plants subjected to temporary drought conditions (*Fig. 4*). However, the relative water content in leaves changed more significantly under the combined action of rhizobacteria and AS in the plants subjected to low moisture supply (35% WHC) compared to the variant with the use of *B. japonicum* alone. The positive effect obtained due to the application of the bacterial strains could be conditioned by the hydraulic nature of the root system, which was better developed, with more fasciculate roots, improving radial water transport, as demonstrated in the research of Kothari and authors (1990). Likewise, it should be mentioned that the improvement of water status in plants could be explained by the fact that the application of rhizobacteria obviously enhanced the development of the root system, because in the literature was reported that bacterial strains have the ability to produce phytohormones, especially AIA (3-acid indolyl acetic). Therefore, it can be assumed that the application of rhizosphere microorganisms has created additional opportunities for the exploration of a larger soil volume and more efficient utilization of soil moisture reserves. In general, water deficit associated with insufficient phosphorus in soil contributes to a decrease in plant water content and this had negative effects on soybean productivity (data not shown). Therefore, the results of this study contribute, to some extent, to understand profoundly the necessity of the integrated use of rhizobacteria and salicylic acid for enhancing plant cross-tolerance to phosphorus deficiency and moisture deficit of soil.

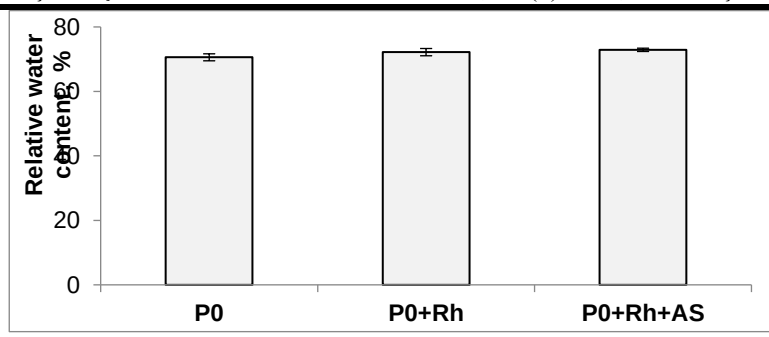

Fig. 4. The influence of *B. japonicum* and AS on relative water content in leaves in soybean grown under low P and low soil moisture (35% WHC)

The integrated application of beneficial microorganisms and acid salicylic could offer an alternative to improve plant nutrition and growth under abiotic stresses. However, further studies are required to investigate the effect of rhizobacteria and AS on physiological changes of plants for long period of abiotic stresses.

CONCLUSIONS

1. The present study indicated that phosphorus insufficiency and low soil humidity caused changes in nutrient status of soybean leaves.
2. The integrated use of the rhizobacteria *B. japonicum* and salicylic acid improves phosphorus and potassium nutrition of plants under both optimal moisture and temporary low soil moisture.
3. The combined application of rhizobacteria B. japonicum and salicylic acid maintains higher leaf water content in soybean plants subjected to both phosphorus deficiency and moderate drought. Therefore, using rhizobia and salicylic acid together could enhance phosphorus and potassium nutrition in soybean under conditions of low P and water deficit.

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