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EFFECT OF *Bradyrhizobium japonicum* AND SALICYLIC ACID ON PHOTOSYNTHETIC PIGMENTS CONTENTS AND SOYBEAN GROWTH UNDER PHOSPHORUS AND WATER DEFICIT CONDITIONS

Vladimir ROTARU

Institute of Genetics, Physiology and Plant Protection, 20 Padurii Street, Chisinau, Republic of Moldova

Corresponding author email: rotaruvlad@yahoo.com

Abstract

The application of rhizobacteria and salicylic acid is a promising approach in crops production and they can induce resistance against abiotic stress, however, their combined effect is not fully elucidated. A pot experiment was conducted to study the effect of rhizobacteria Bradyrhizobium japonicum applied alone or in combination with salicylic acid (SA) on photosynthetic pigments (chlorophyll a, b, total chlorophyll, and carotenoids) and plant growth of soybean under low phosphorus and drought conditions. The plants exposed concomitantly to low P and temporary water deficit registered the lowest growth and photosynthetic pigments status. Integrated use of rhizobacteria and SA (0.5 mM) increased photosynthetic pigments contents in leaves under normal as well as under water deficit conditions. Their effect was more pronounced in plants subjected to P deficiency and water stress. Results showed that seed treatment with rhizobacteria in conjunction with foliage application of salicylic acid increased plant growth irrespective of soil moisture level. It is inferred that rhizobacteria and SA work synergistically to promote growth of plants under moisture and nutrient deficit conditions.

Key words*: chlorophylls, drought, phosphorus, rhizobacteria, salicylic acid, soybean.*

INTRODUCTION

Drought and insufficiency of phosphorus (P) are major abiotic environmental factors, that often coexist in field conditions and they have significant adverse effects on the growth and productivity of crops. Water deficit stresses are the global issues that affect water and nutrient relations, assimilate production, photosynthesis and respiration in plants. In addition, P-deficiency affects a wide range of physiological, metabolic and biochemical processes in plants (Fredeen et al., 1989; Gan et al., 2002). Legumes, particularly soybean, play an important role in the development of organic and sustainable agriculture. Soybean is the most important source of vegetable oil in the world. However, this species is very sensitive to low P as well as to drought, especially during reproductive development stage (Silvente et al., 2012). This crop is severely affected by drought, resulting in major yield losses particularly in the drought prone areas of Eastern and South Europe countries. In soybean plants, water deficit stress reduces photosynthetic pigments accumulation, biomass production and finally the grain yield (Hossain et al., 2014; Tripathi et al., 2015). Thus, there is a need to find out strategies to maintain the growth and physiology activity of plants grown under unfavorable environmental conditions at more or less acceptable levels.

There is a body of scientific information regarding the beneficial effects of rhizobacteria *Bradyrhizobium japonicum* sp on soybean growth and yields (Abbasi et al., 2013). This bacteria species has many plant growth promoting activities. Along plant growth promoting rhizobacteria, plant growth regulators (PGRs) also have a vital role in maintaining many processes at physiological levels with beneficial repercussions on crops productivity (Asgher et al., 2015). Many reports have documented that exogenous application of plant growth regulators can mitigate the abiotic stress-induced inhibitory effects on plant growth and productivity (Ashraf et al., 2010). Among PGRs, salicylic acid (SA) was demonstrated to be a messenger involved in signal transduction in response to biotic and abiotic stresses (Kim et al., 2018; Raskin, 1992). Salicylic acid acts as an endogen phytohormone from phenol compounds, involved in the antioxidant defense system and regulates various physiological and biochemical parameters of plants such as stomata conductivity (Hayat et al., 2010; Khan et al., 2015; Raskin, 1992), activity of photosynthetic pigments (Hayat et al., 2010), maintenance of tissue water contents and reduced membrane permeability (Farooq et al., 2009) and tolerance to environmental stresses (Kabiri et al., 2014; Khan et al., 2018). Khodary (2004) observed a significant increase in growth, pigment contents and photosynthetic rate in maize, sprayed with salicylic acid. Some researchers demonstrated that the exogenous application of SA at lower concentrations within the range of 0.1-0.5 mM improves photosynthesis, growth and various physiological and biochemical processes, whereas in higher concentrations, more than 1 mM, SA may cause stress in plants (Hayat et al., 2010). Taking into consideration that on one hand, the application of *B. japonicum* strain has contributions to promote plant growth of soybean, and on the other hand salicilic acid may alleviate some negative effects of drought, therefore the main objective of this study was to examine if combined use of rhizobacteria and salicilic acid has synergic effect on photosynthetic pigments contents and plant growth of soybean plants subjected concomitantly to P insufficiency and water deficit conditions.

MATERIALS AND METHODS

A greenhouse experiment was conducted in controlled water conditions to examine the effect of *B. japonicum* application alone or in combination with foliage treatments with salicylic acid on photosynthetic pigments contents and plant growth of soybean (*Glycine max* L. Merrill) under low P supply and temporary water deficit conditions.

Seeds of soybean (cv Horboveanca) were sown in plastic pots (10 L) and filled with soil and sand in a ratio of 3:1. The soil used for the experiment was chernoziom carbonated. Before sowing, the soybean seeds were thoroughly mixed with *B. japonicum* inoculants (10^8 cfu/mL). There were 4 replicate pots per treatment, with two plants per pot. All plants from each treatment were grown under normal soil moisture until the flowering (R1) stage. Normal irrigation was maintained at 70% WHC (water holding capacity). Half of the plants were subjected to water deficit (35% WHC) at full flowering stage for 12 days. Plants were treated with SA (0.5 mM) at the branching and blooming stages. Control plants were not treated with rhizobacteria and salicylic acid. The experiment was in a completely randomized design and four replications were performed for flowering setting pods stage and four for maturity grain stage. Plants were collected after drought stress period and analyzed. Determination of photosynthetic pigments (chlorophyll *a, b*, total chlorophylls and carotenoids) was done using the method of Arnon (1949). Using acetone (80%, v/v) the fresh leaves were homogenized and the supernatant was read at 663, 645 and 452 nm. Values were presented as means with standard errors (SE) from three independent treatments. The differences in the means were determined by the least significant difference (LSD) (P = 0.05) test. Data were statistically analyzed using analysis of variance (ANOVA) by Statistic 7 program.

RESULTS AND DISCUSSIONS

Plant growth regulators, like salicylic acid (SA), are chemical compounds produced in one part of plant and translocated to the other parts, where they play important roles in regulating plant responses to any kind of environmental stresses. Phytohormones play an important role in influencing drought tolerance through modulating several physiological processes and different protective mechanisms. The role of SA has been reported as being critical in modulating physiological responses that lead to the adaptation of plants to unfavorable environments (Nazar et al., 2011; Kang et al., 2013). It is worthy to note that the combined effect of rhizobacteria *B. japonicum* and SA on pigments contents and plant growth in soybean subjected together to P deficiency and moderate drought has not been reported.

Salicylic acid and bacteria strains were found to induce significant effects on various physiological changes in plants. In this study, it was observed that exogenous application of SA enhanced the growth and photosynthetic pigments contents under normal irrigation condition (Figure 1) as well as under drought stress (Figure 2).

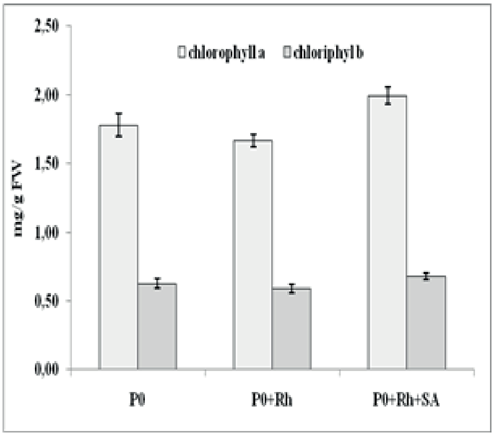

Figure 1. Effect of *B. japonicum* and salicylic acid on content of chlorophyll *a, b* in soybean leaves under low P and normal irrigation, 70% WHC. Data are presented as treatments mean ± SE (n = 4). P0-low phosphorus, Rh-*B. japoniucm,* SA-salicylic acid

The effects of rhizobacteria *B. japonicum* and SA on photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) contents of soybean plant are presented in Figures 1 and 2 and in Table 2. Experimental results revealed that rhizobacteria and salicylic acid had stimulatory effects on photosynthetic pigments contents in soybean plants. The drought decreased significantly the photosynthetic pigments concentrations in all treatments (Figure 2) compared to control plants (70%WHC). It was revealed that the content of chlorophyll *a* and *b* significantly decreased by 94-114% and 47.5-61.5%, respectively (Figure 2), in the leaves of drought stressed plants than in those under normal irrigation (Figure 1). However, plants treated with *B. japonicum* in combination with SA showed lower reductions compared to untreated plants grown under drought stress. Our results are in agreement with those of Mafakheri et al. (2010) and Pospisilova et al. (2011) who reported that exogenous application of SA increased the proline content under the conditions of drought stress. The application of rhizobacteria *B. japonicum* sp alone was also effective in improving the chlorophyll status of leaves. However, the combined use of bacteria strain and SA displayed the better result.

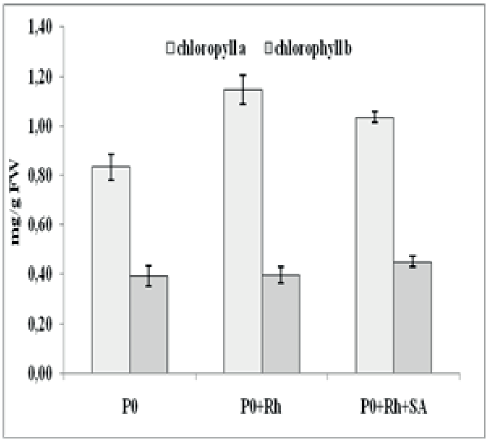

Figure 2. Effect of *B. japonicum* and salicylic acid on content of chlorophyll *a, b* in soybean leaves under low P and water deficit conditions, 35% WHC. Data are presented as treatments mean ± SE (n = 4). P0-low phosphorus, Rh-*B. japoniucm,* SA-salicylic acid

Therefore, the foliar application of plant growth regulator SA on combination with bacteria strain significantly increased the chlorophyll content in soybean plants. The beneficial effect of SA on photosynthetic pigments in our study is in accordance with those obtained by Barakat (2011) on wheat and Saeidnjad et al. (2012) on maize. Similar to our findings, exogenous application of SA increased the chlorophylls content of soybean leaves as reported by Khan et al. (2003). Foliar application of SA (0.5 mM) enhanced pigment content by about 18% for total chlorophyll (Table 1) and by 27.6% for carotenoids (Table 2) at the end of drought stress compared to untreated plants. Hence, in our research, drought stress caused the reduction of photosynthetic pigments but rhizobacteria and SA foliage treatment increased green pigments and carotenoids contents in water stress conditions. Regarding SA effect, Khodary (2004) and Szepesi (2006) found that SA treatment increased the chlorophyll, carotenoids contents, improved the photosynthetic efficiency and enhanced photosynthetic rate, the dry weights and maintained membrane integrity, leading to improvement of plant growth in maize and tomato plants respectively under stress conditions. The experimental results of the present study revealed that total chlorophylls in leaves increased by 11.7% as a result of combined use of rhizobacteria and SA in plants cultivated at normal level of soil moisture (Table 1). However, their application was more efficient in plants subjected to water deficit, and total chlorophylls increased by 18% compared to control plants.

Table 1. Total chlorophylls content (mg/g FW) in soybean leaves in relation to *Bradyrhizobium japonicum* and salicylic application under low phosphorus and water deficit condition. P0-low phosphorus, Rh-*B. japoniucm,* SA-salicylic acid

Treatments	70% WHC		35% WHC	
	Total chlorophylls mg/g	Ration a/b	Total chlorophylls, mg/g	Ration a/b
P0	2.40	2.82	1.23	2.13
P0+Rh	2.26	2.83	1.54	2.87
P0+Rh+SA	2.68	2.94	1.49	2.29

It was documented that carotenoids are involved in processes of reactive species oxygen annihilation, in photosynthetic machinery stabilization, hence contributing to diminish the adverse effects of abiotic factors on plants (Moharekar et al., 2003). Therefore, it was interesting to evaluate the changes of carotenoids contents in leaves in relation to application of rhizobacteria alone or in combination with SA. The application of *B. japonicum* separately or in combination with SA did not significantly affect their concentrations in plants cultivated under both soil moisture levels. Under drought stress, reduction of carotenoids could be related to its protection role in the photosynthetic apparatus, because carotenoids were responsible for scavenging of ROS, preventing lipid oxidation, and ultimately the mitigation of oxidative stress (Moharekar et al., 2003). Applied SA may temporarily lower the level of oxidative stress in plants, which acts as a hardening process, improving the antioxidative capacity of plants and helping to induce the synthesis of protective compounds (such as carotenoids) (Kim et al., 2018; Hayat et al., 2010).

The carotenoids content showed variable results only in plants subjected to temporary water deficit. There were not significant changes of this physiological parameter in plants grown under normal irrigation condition. However, the application of rhizobacteria increased this parameter significantly in leaves of soybean plants grown under water deficit conditions (Table 2). Similar effect was observed in treatment with combined use of rhizobacteria and SA. It is worthy to note that biomass of leaves in that treatment registered higher value than in treatment with bacteria strain separately.

Table 2. Carotenoids contents in soybean leaves under application of rhizobacteria and salicylic acid in plants grown under low phosphorus and water limited conditions, mg/g FW. P0-low phosphorus, Rh-*B. japoniucm,* SA-salicylic acid

Treatments	70% WHC	35% WHC
P0	0.82±0.01	0.322±0.11
P0+Rh	0.68±0.03	0.413±0.23
P0+Rh+SA	0.77±0.02	0.411±0.18

The application of rhizobacteria and plant growth regulators is an effective approach for improving plant productivity. Value of biomass production is considered a summary parameter of PGPR and plant growth regulators efficiency. Generally, plants cultivated concomitantly under low P and water deficit registered the lowest productivity. However, plant growth promoting rhizobacteria applied alone or in conjunction with SA treatment significantly increased plant biomass (Figure 3). Under water deficit conditions, the highest increase (39.1%) in plants growth was recorded in combined treatment of rhizobacteria and SA compared to control. It is necessary to mention that water status of soybean plant was reduced under drought stress, while SA treatments improved leaf water status (data not shown) enabling the plants for efficient water intake resulting in a positive influence on photosynthetic machinery and as a result plant growth enhanced.

Likewise, this beneficial effect was registered in plants cultivated under normal irrigation condition. The rhizobacteria applied alone had less effect on plant dry weights as compared to combined application of bacteria strain and SA. It is necessary to note that drought stress reduced the root dry weights significantly in all plants, however, combined treatment of *B. japonicum* and SA significantly reduced the negative influence of drought stress and low P on roots growth (data not shown).

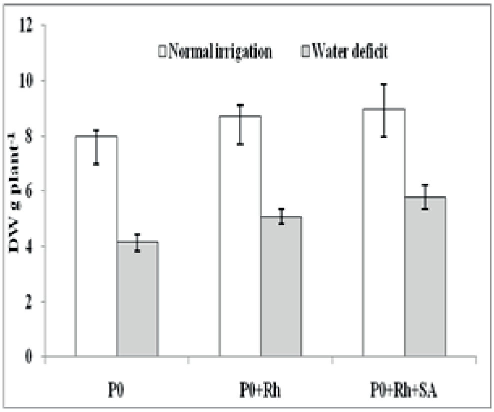

Figure 3. Effects of rhizobacteria and SA on soybean growth under low P and different soil moisture conditions. Data are presented as treatments mean ± SE (n = 4)

Arfan et al. (2007) revealed that foliar application of salicylic acid increased grain yield and performance of spring wheat plants subjected to water deficit conditions. The experimental results proved the role of PGPR and SA in improving the tolerance of plants and suggested that rhizobacteria and SA could be a useful approach to improve photosynthesis and plant growth.

Thus, enhancement of the level of chlorophylls and carotenoids pigments as well as the plant growth demonstrated the synergic interaction assigned to salicylic acid and *Bradyrhizobium japonicum* bacteria.

CONCLUSIONS

The application of rhizobacteria *Bradyrhizobium japonicum* and foliar application of 0.5 mM salicylic acid improved photosynthetic pigments status of soybean plants under normal and temporary water deficit conditions. The combined use of *B. japonicum* strain and SA treatments had synergic effect on growth parameters of soybean under low phosphorus and water deficit conditions. Overall, the results indicated that integrated use of rhizobacteria and salicylic acid could be used as a biological approach to enhance plant tolerance to environmentally unfavorable conditions.

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