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BIOFERTILIZER BASED ON SILICATE SOLUBILIZING BACTERIA IMPROVES PHOTOSYNTHETIC FUNCTION OF *BRASSICA JUNCEA*

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ABSTRACT

Usage of biofertilizers is one of the important components of integrated nutrient management, as they are renewable source of plant nutrients, ecologically safe compared to chemical fertilizers and cost effective. Silicate solubilizing bacteria (SSB) can play an efficient role not only in solubilizing insoluble forms of silicates but also potassium and phosphates, hence increasing soil fertility and thereby enhancing plant productivity. The aim of this study was to investigate the influence of SSB-enriched biofertilizer on the structural and functional parameters of photosynthetic apparatus of *Brassica juncea* (L.) Czern. The pure culture of SSB was isolated from the clay substrate, cultivated on Zak–Alexandrov medium and identified as *Bacillus sp.* To obtain the biofertilizer, SSB culture (0.6_*10^8 cfu mL^{-1}) was mixed with steriled peat (1:1, v/w) and dried at 35–40°C. Plants were grown from seeds during two months in the pots with adding SSB-enriched biofertilizer to the mixture of clay and soil (1:10, w/w). The clay substrate plus peat without SSB was used as a control. It was found that addition of SSB-enriched biofertilizer to clay substrate significantly increased the content of total nitrogen, phosphorus and potassium in the leaves of *B. juncea*. The thickness of mesophyllic layer and the number of mesophyll cells were increased on average by 24 %. It was correlated with a sharp increase of photosynthetic pigment content and CO_2 uptake (1.5–2.0 times). We can conclude that SSB-enriched biofertilizer improves the photosynthetic function of *B. juncea*.

Keywords: *biofertilizer, silicate solubilizing bacteria, Indian mustard, photosynthesis.*

INTRODUCTION

At present, the production of a sufficient number of “ecologically clean” food products is one of the global challenges facing humanity (Dubey and NidhiShukla, 2014). To satisfy the demand in food and increase the productivity of crops some non-environmentally friendly technologies are commonly been used such as pesticides and synthetic fertilizers, which can cause health problems. An alternative to this is organic agriculture which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. This is accomplished by using new safe technologies, such as the usage of biofertilizers (Aggani, 2013; Malusà *et al.*, 2016). Biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients, ecologically safety instead of chemical fertilizers (Raja, 2013). Different kinds of promoting plant growth microorganisms (PGPM) can be used for the production of biofertilizers. Silicate solubilizing bacteria (SSB) can play an efficient role not only in solubilizing insoluble forms of silicates but also potassium and phosphates, hence increasing soil fertility and enhancing plant productivity (Han and Lee, 2005). Phosphate and potassium are major essential macronutrients for plant growth and development and soluble P and K fertilizers are commonly applied to replace removed minerals and to optimize yield. This is especially important for the reclamation of infertile or disturbed soils that are not suitable for sustainable agriculture. Numerous studies showed the effect of SSB on the nutrient uptake from the soil, their positive influence on photosynthesis and the growth of some crops (Han and Lee, 2005; Han *et al.*, 2006; Tripti *et al.*, 2017). However, the structural characteristics of the photosynthetic apparatus, that provide plant productivity, practically were not investigated. The aim of this study was to investigate the influence of SSB-enriched biofertilizer (SSB-EB) on the structural and functional parameters of photosynthetic apparatus of *Brassica juncea* (L.) Czern.

MATERIAL AND METHODS

The pure culture of SSB (*Bacillus sp.*) was isolated from the clay substrate taken from the mountain territory of Middle Urals (Russia), and cultivated on Zak–Alexandrov medium in following composition (g L^{-1}): $MgSO_{4*}7H_2O$ – 0.15 g, NaCl – 0.15 g, $MnSO_4$ – 0.05 g, $FeSO_{4*}7H_2O$ – 0.05 г, Na_2SiO_3 – 2.0 g, $CaCO_3$ – 2.0 g, $Ca_3(PO_4)_2$ – 1.5 g, KNO_3 – 1.0 g, sucrose – 20 g, agar – 15 g. The isolate was identified by morphological and biochemical test followed by Bergey's manual (Bergey *et al.*, 1984). To obtain the biofertilizer, SSB culture ($0.6{*}10^8$ cfu mL^{-1}) was mixed with purified sterile peat (1:1, v/w) and dried at 35–40°C.

Brassica juncea was grown from seeds in the pots with adding SSB-EB to the clay soil (1:10, w/w). The clay substrate plus peat without SSB were used as a control. After two months, mature leaves were used for the physiological experiments. The content of total nitrogen (N) and phosphorus (P) in *B. juncea* leaves was determined with a PD-303 UV spectrophotometer (Apel, Japan) after wet digestion with the mixture of acids H_2SO_4 and $HC1O_4$ as described earlier (Borisova *et al.*, 2014). The content of potassium (K) was determined using the atomic absorption spectrometry (AAS Vario 6, "Analytik Jena", Germany) after wet digestion with 70 % HNO_3 (analytical grade). Leaf mesostructure parameters were determined in 20 replicates according to Ivanova and P'yankov (2002). Transverse slices of leaves were obtained using a freezing microtome MZ-2 (Russia). The thickness of leaf was measured by inspecting the leaf cross sections with a Meiji Techno light microscope (Japan) and an eyepiece micrometer AM 9-2 (GSZ, Russia). A computer-assisted protocol based on Simagus Mesoplant software (OOO Siamz, Russia) was used to determine the quantitative parameters of mesophyll cells. Leaves were preliminary macerated with 5% chromic acid dissolved in 1 N HCl. The pigment content was spectrophotometrically determined in 96 % ethanol according to Lichtenthaler (1987). The photosynthetic rate was measured as CO_2 uptake with infrared gas analyzer (LI-COR, USA). All data were analyzed statistically by analysis of variance using Statsoft Statistica 7.0. Data are presented as mean (n = 3–20) ± standard error (SE). As the most variables did not fit a normal distribution, the differences between mean values were calculated using nonparametric Mann–Whitney U-test at $p < 0.05$.

RESULTS AND DISCUSSION

Brassica juncea (L.) Czern., commonly known as Indian mustard, is an important agricultural crop in different parts of the world. It is used for oil production and as a condiment, has medicinal properties. Plants grow fast and produce large amount of above-ground biomass. In addition, they can accumulate large amount of heavy metals and accordingly been used for phytoremediation of disturbed ecosystems (Shekhawat *et al*., 2012; Singh and Fulekar, 2012). It was found that addition of SSB-EB to clay substrate significantly increased the content of studied biogenic elements in the leaves of *B. juncea* (Table 1). The amount of total nitrogen, phosphorus and potassium increased by 18, 20 and 25 %, respectively, as compared to the control. Such nutrients are required in large quantities and involved in almost all methabolic reactions in crop plants. It is known that nitrogen and phosphorus play an important role in the plant anabolic and catabolic processes.

Table 1. The content of total nitrogen, phosphorus and potassium in *B. juncea*, growing two months without and with adding SSB-enriched biofertilizer.

Variant	Total nitrogen, % DW	Total phosphorus, % DW	Total potassium, % DW
Control	3.20 ± 0.03	0.15 ± 0.01	3.47 ± 0.02
SSB-EB	3.78 ± 0.04*	0.18 ± 0.01*	4.33 ± 0.04*

Data present the mean ± standard error. Asterisks indicate significant differences from control (n = 3, $p < 0.05$).

On the other hand, potassium promotes root growth and increases resistance to cold and water stress. It directly connects with improvement of the quality of crop, reduces pest and disease incidence by enhancing crop resistance as well. Although K is not a constituent of any organic molecule or plant structure, such as N and P, it is involved in numerous biochemical and physiological processes and play an important role for plant growth, yield, quality and stress tolerance (Cakmak, 2005).

A similar trend of increase in NPK uptake by pepper and cucumber at co-inoculation with phosphate and potassium solubilizing bacteria was observed in the study of Han *et al.* (2006). The structure and functional activity of the photosynthetic apparatus should be investigated at different levels of its organization. Plants uptake nutrients from the soil by the roots, transporting them to the leaves. Variations in the macronutrient composition lead to the changes in the leaf mesostructure (Table 2), as an essential exhibition of the photosynthesis regulation at the morphogenetic level.

Table 2. The mesostructural parameters of *B. juncea* leaf: thickness of leaf and its epidermal and mesophyllic layers (A); characteristics of mesophyll cell (B) and chloroplast (C).

A

Variant	Leaf thickness, μm	Epidermis thickness, μm	Mesophyll thickness, μm
Control	183.1 ± 7.6	49.3 ± 2.6	133.8 ± 5.9
SSB-EB	197.6 ± 6.9	41.7 ± 2.0	155.9 ± 6.4*

B

Variant	Cell surface area (S), thousand μm^2	Cell volume (V), thousand μm^3	Number of cells per unit area, thousand sm^{-2}
Control	3.2 ± 0.2	14.5 ± 1.0	128.2 ± 2.4
SSB-EB	2.2 ± 0.1*	8.5 ± 0.5*	167.6 ± 1.5*

C

Variant	Chloroplast surface area, μm^2	Chloroplast volume, μm^3	Number of chloroplasts per unit area, million sm^{-2}
Control	68.4 ± 4.6	55.0 ± 5.6	3.6 ±0.1
SSB-EB	63.9 ± 2.5	48.6 ± 2.9	4.9 ±0.0*

Data present the mean ± standard error. Asterisks indicate significant differences from control ($n = 20$, $p < 0.05$).

The *B. juncea* leaf thickness was increased due to a significant increase of the thickness of mesophyllic layer (Table 2A). At the same time, the decrease in the mesophyll cell surface area and volume was compensated by the increase of their number per unit area – by 30 % of control (Table 2B). A similar trend was observed in chloroplasts, a decrease in the surface area and volume of these organelles was accompanied by the increase of their number per unit area, which led to growth of plastid material volume (Table 2C).

The ratio of the cell surface area to its volume (*S/V*) affects the intensity of the gas exchange. According to Fick's law for passive diffusion of gases, the time for which the concentration of CO_2 in the cell increases from zero to half the concentration in the external medium is inversely proportional to the ratio *S/V*

(Niklas, 1997). It was found that growing *B. juncea* with adding SSB-EB to clay substrate significantly increased this ratio (by 16 %). The larger the ratio of the cell surface area to the volume, the higher the diffusion rate of CO_2 from the intra-leaf space to the chloroplasts.

It is known that the content of photosynthetic pigments can significantly affect the functioning of the photosynthetic apparatus and the metabolism of the whole plant. Their amount was found increased in the leaves of *B. juncea*, grown with adding of SSB-EB (Fig. 1A).

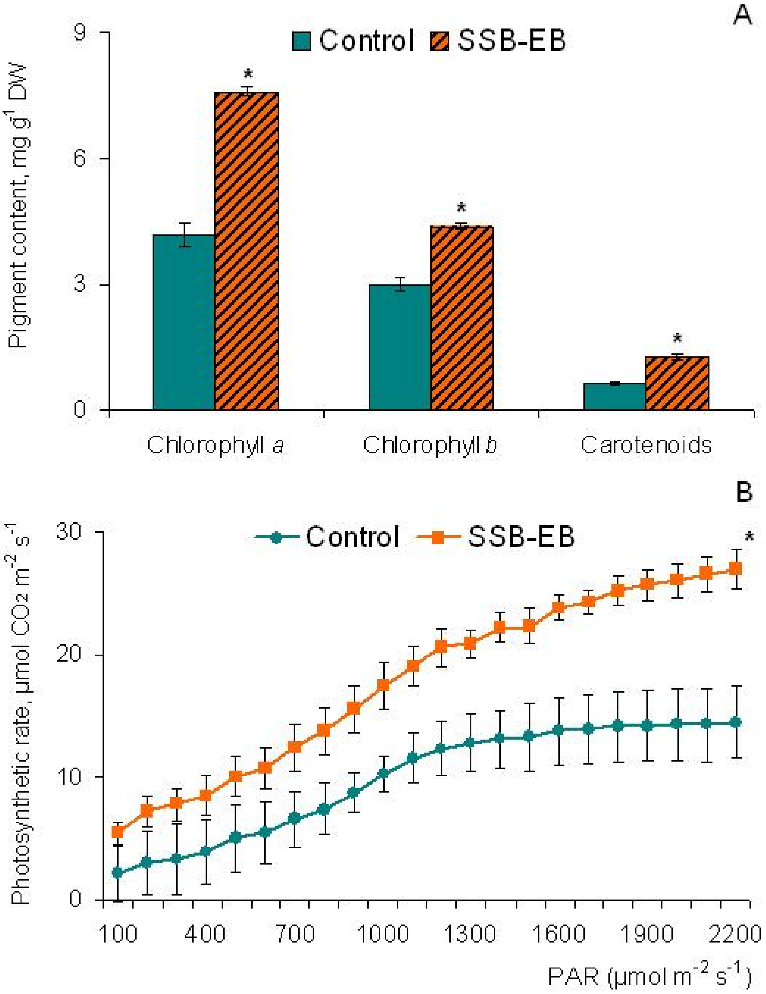

Figure 1. The content of photosynthetic pigments (A) and photosynthetic rate (B) in the leaves of *B. juncea*, growing two months without and with adding SSB-enriched biofertilizer. Data present the mean ± standard error. Asterisks indicate significant differences from control (n = 3, $p < 0.05$).

The content of chlorophyll *a* increased more significantly (by 82 %) than chlorophyll *b* (by 46 %). The content of carotenoids raised almost 2 times as compared to the control. The increase of chlorophyll amount in the mustard grown with adding SSB-EB can obviously be explained by an increase in the content of total nitrogen in the leaves, since it participates in the construction of these pigments. A sharp increase of photosynthetic pigment content correlated with CO_2 uptake ($p < 0.01$). The rate of photosynthesis was almost 2 times higher in experimental plants (Fig. 1B).

Photosynthesis is one of the leading plant functions, which ensures their growth, development and productivity. Changes in the structural and functional parameters of the photosynthetic apparatus are a reflection of morphogenetic regulation in plants.

CONCLUSION

The adding SSB-enriched biofertilizer to clay substrate significantly increased the thickness of mesophyllic layer, the number of mesophyll cells, the plastid material volume and the photosynthetic pigment content in the leaves of *B. juncea*. This led to enhanced CO_2 uptake by Indian mustard. Consequently, we can conclude that biofertilizer based on silicate solubilizing bacteria improved the photosynthetic function of *B. juncea*.

Changes in the studied parameters of mustard plants grown with adding of SSB-EB can be regarded as the result of increasing the available forms of macronutrients content in substrate due to the solubilization of clay silicates. This is confirmed by enlargement of the total phosphorus and potassium content in the leaves of *B. juncea*.

Hense, the bioformulation with adding SSB can be used as effective eco-friendly fertilizer for reclamation of infertile or disturbed lands, as well as for increasing the productivity of crops in organic agriculture with minimal costs.

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