

Short Title: Biochemical characteristics of corn grain under the presowing seed treatment with rhizobacteria *Paenibacillus polymyxa* KB and chelate complex of biogenic silicon

RESEARCH ARTICLE

Biochemical characteristics of corn grain under the presowing seed treatment with rhizobacteria *Paenibacillus polymyxa* KB and chelate complex of biogenic silicon

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Abstract

We used corn seeds of maize (*Zea mays* L.) of the Dnieper 196 SV hybrid to analyse presowing seed bacterization and treatment with biogenic silicon that could be promising in growing maize hybrids. Using presowing seed bacterization and treatment with biogenic silicon resulted in the total protein and fibre content increasing by 3 times-4 times and 21-27%, respectively. The mono- and disaccharide content increased by 2-3 times and the starch content decreased up to 10%-20% in corn grains under treatment with both preparations. During presowing seed bacterization, malondialdehyde content increased, whilst the ascorbic acid content, catalase and ascorbate peroxidase activity, and antioxidant activity reduced. Using presowing seed treatment with BSi resulted in the malondialdehyde content decreasing and the activity of catalase and ascorbate peroxidase increasing. This agricultural method leads to an increase in the protein, carbohydrate, and fibre content, which is valuable in terms of their nutrition. Both variants of presowing seed treatments are promising in growing maize hybrids.

Keywords: Corn grain, rhizobacteria, biogenic silicon, presowing seed treatment, saccharides, starch, malondialdehyde, catalase, ascorbate peroxidase

Introduction

Maize (*Zea mays* L.) is one of the most common and important cash crops in the world (Galindo et al., 2020). Different factors influence on the grain quality of this crop. Different agrotechnologies can involve the use of plant growth regulators that have a complex effect on growth and development (Galindo et al., 2020; Panuccio et al., 2018). Seeds are a major and vital component of sustainable agricultural productivity growth. Therefore, one of the effective ways to influence the processes of plant growth and development and the formation of resistance to various environmental stressors are the presowing seed treatment with different biologically active substances or its combinations. The aim of the present work is to investigate the effect of microbial preparation of plant growth promoting rhizobacteria and chelate complex of Biogenic Silicon (BSi) on biochemical characteristics of corn grain.

Materials and Methods

This study was conducted in Nizhyn, Chernigiv region, Ukraine. Seeds of corn (*Zea mays* L.), hybrid Dniprovsky 196 SV were used in the present study. Before sowing, the seeds were treated with the studied preparations. The experiment used the microbial preparation Polymyxobacterin (Institute of Agricultural Microbiology and Agro-industrial Production of NAAS, Chernigiv, Ukraine), based on the active growth promoting rhizobacteria, *Paenibacillus polymyxa* KB (*P. polymyxa*; TU U 24.1-00497360-004:2009). Bacterization of corn seeds (1 ml per 300 g of seeds) was performed according to SOU 01.11-37-783. Chelate complex of BSi is a biologically active immunoprotector (BAI-SI) ("Agroecotech" Ltd., Kyiv, Ukraine) based on silicon (active substances: silicon in ionic form (silicon oxide), 5%-7%; potassium oxide, 2.2%-3.3%; and trace elements including: iron, copper, and zinc). Seed (300 g) presowing with chelate complex of BSi was performed in doses of 25 ml L⁻¹ of water. The processing time of corn seeds

was 1 hour. After seed treatment, corn was sown in a square-nested manner in the field soil. The soil cover of the experimental field is chernozem podzol, low humus. The total area of the sown area was 88 m². The study was arranged as a completely randomized design with 3 groups and three replicates: control, seed bacterization and seed treatment with BSi. Field trials in our work were repeated during 2017-2019. Corn grain protein content determination was done according to (Lowry et al., 1951). Quantification of soluble sugars and starch content in corn grains were carried out with anthrone reagent following the method used by (Dien et al., 2019). The "raw" fiber was done according to (Bala et al., 2013). The content of MDA in the corn grains were measured by the 2-thiobarbituric acid method (Hasanuzzaman et al., 2012). The ascorbic acid content was determined according to (Alam et al., 2014). Ascorbate peroxidase (EC 1.11.1.11) and catalase (EC 1.11.1.6) activities were measured, respectively, according to (Nakano & Asada, 1981; Aebi, 1984). The total antioxidant activity of corn grain homogenates was calculated as the percentage inhibition of adrenaline autooxidation (Barkovsky, 2013). Carotenoid concentrations were calculated according to (Sumanta et al., 2014). The data was analysed with the computer-based software XLSTAT following analysis of variance. A Student's t-test was used for the statistical analysis. Differences in values were considered statistically significant at $p \leq 0.05$. The results are expressed as the mean and standard deviation (SD) of the mean. Mean (\pm SD) was calculated from three replicates for each treatment.

Results and Discussion

Presowing seed bacterization and treatment with BSi increased the total protein content in corn grains by 2.6 times and 3.7 times, respectively, as relative to control (Tab. 1). This increase in protein content under the different variants of presowing seed treatments can be explained by the fact that the microbial preparation Polymyxobacterin includes phosphate-mobilizing bacteria, *P. polymyxa*, which are active producers of exopolysaccharides (Premachandra et al., 2016). These polysaccharides increase the content of guaiacol-dependent and o-phenylene-dependent peroxidases in cells, which may stimulate protein synthesis in plant cells. Whereas the components of the chelate complex of the vital BSi, in the form of an aqueous solution, may activate protein biosynthesis.

Table 1. Effect of seed bacterization and seed treatment with BSi on total protein, carbohydrates, fiber content, some in dices of lipid peroxidation and antioxidant system in corn grains, 2017-2019.

	Control	Seeds bacterization	Seeds treatment with biogenic silicon
Total protein content (mg/g FW)	1.48 \pm 0.51	3.98 \pm 0.51*	5.56 \pm 1.50*
Monosaccharides content (mg/g FW)	139.00 \pm 65.87	410.00 \pm 31.03*	304.80 \pm 38.81*
Disaccharides content (mg/g FW)	261.44 \pm 91.87	467.02 \pm 83.31*	627.76 \pm 137.07*
Starch content (mg/g FW)	594.67 \pm 36.49	532.33 \pm 31.06	496.67 \pm 33.89
Fiber content (% dry weight basis)	2.24 \pm 0.18	2.72 \pm 0.27*	2.84 \pm 0.24*
MDA (μ mol/g FW)	2.14 \pm 0.06	2.47 \pm 0.08*	1.32 \pm 0.11*#
Ascorbic acid content (mmol/g FW)	0.027 \pm 0.0050	0.016 \pm 0.0008*	0.029 \pm 0.010
Ascorbate peroxidase activity (μ mol ascorbate /g FW min ⁻¹)	1.196 \pm 0.017	0.721 \pm 0.136*	2.893 \pm 0.821#
Catalase activity (μ cat/g FW)	2.39 \pm 0.01	1.83 \pm 0.08*	2.59 \pm 0.46
Total antioxidant activity (%)	82.92 \pm 0.60	76.82 \pm 1.21*	81.71 \pm 3.66
Total carotenoids content (mg %)	2.15 \pm 0.26	2.10 \pm 0.13	2.47 \pm 0.37

* Means in a column with asterisk are significantly different ($p \leq 0.05$) compared to control. # Mean in a column with # are significantly different ($p \leq 0.05$) compared to group with seeds bacterization.

The water-soluble sugars (mono and disaccharides) in corn grains increased in both experimental groups. Thus, the monosaccharides content in corn grains increased by 2.9 times and 2.2 times and the disaccharides increased by 1.8 times and 2.4 times following presowing seed bacterization and treatment with BSi, respectively, compared to the control. The stimulating effect of presowing seed bacterization in relation to this indicator can be explained by the fact that *P. polymyxa* bacteria in the rhizosphere of plants convert complex soil compounds into simple ones available for plant nutrition, increasing the size of the root system and improving phosphorus nutrition (Premachandra et al., 2016). Organic silicon from the chelate complex of BSi is involved in the process of carbohydrate phosphorylation. This enhances the synthesis of simple sugars and helps to increase the sugar content of corn grains. At the same time, the starch content in corn grains decreased by 10.5% and 16.5% following presowing seed bacterization and treatment with BSi compared to the control, which can also lead to an increase in the content of mono- and disaccharides. The fiber content in the control corn grain was found to be 2.24%. Presowing seed bacterization and treatment with BSi showed a high efficiency and increased the fiber content by 21% and to 27% in comparison with the control. Our results showed that MDA content in maize grains, as a product of lipid peroxidation, increased by 15% and decreased by 38% following presowing seed bacterization and treatment with BSi compared to the control group. At the same time, changes of functional activity of the antioxidant system, both enzymatic and non-enzymatic, are observed. Ascorbic acid content in corn grains following presowing seed bacterization was 41% lower compared to the control. Subsequently, presowing treatment with BSi, the ascorbic acid content in corn grains did not differ from the control group. Ascorbic acid is considered to be a powerful antioxidant because of its ability to donate electrons in several enzymatic and nonenzymatic reactions. There are several enzyme systems in cells that catalyse the conversion of hydrogen peroxide, including catalase and ascorbate peroxidase. After presowing seed bacterization, the activity of catalase was 23% lower compared to control

and after treatment with BSi was 8% higher and statistically similar to the control. A similar trend was observed for ascorbate peroxidase activity as it was 40% lower compared to the control, and under the presowing treatment with BSi was 142% higher than the control values. The total antioxidant activity in maize grains following presowing seed bacterization was 7% lower compared to the control and did not differ from the control values for presowing seed treatment with BSi. The total carotenoid content in maize grains did not change depending on whether the presowing seed treatment was carried out. After analyzing our results, the inverse relationship between the content of lipid peroxidation products, ascorbic acid, and the activity of ascorbate peroxidase and catalase, as well as with the total antioxidant activity, attracts attention. Such changes in the activity of pro- and antioxidant systems can affect the physiological properties of the grain. Therefore, finding grains at rest is an important adaptive mechanism for species conservation. These specialized systems are involved in the process of rest state formation and can ensure the grain's survival. The viability of the grains is maintained due to the activity of the components of the antioxidant system. The enzyme peroxidase is involved not only in maintaining the viability of grains at rest but is extremely important in their germination (Wang et al., 2019). In dormant grains, peroxidase catalyses the oxidase and peroxidase reactions of various compounds. The reaction product is water, which is needed by the grains at rest to provide the embryo with water. At the same time, the increase in peroxidase activity can initiate the process of seed germination.

Conclusion

Presowing seed bacterization and treatment with BSi lead to an increase in the protein, carbohydrate, and fiber content and improve the quality of corn grains, which is valuable in terms of their nutrition. Presowing seed treatment with BSi also leads to an increase in the antioxidants content in corn grains. Thus, the microbial preparation Polymyxobacterin and chelate complex of BAI-SI are promising preparations when growing maize hybrids.

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