ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 20(3) 2021, 3–13
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2021.3.1
ORIGINAL PAPER
Accepted: 29.05.2020

CHITOSAN AGAINST TO BORON TOXICITY IN MAIZE

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ABSTRACT

In this study; growth, chlorophyll, carotenoid, proline and MDA contents, the amounts of reactive oxygen species (ROS) (superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2)), and antioxidants (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR) activities and its isoenzyme profiles, (ascorbate (AsA), dehydro ascorbate (DHA) and glutathione (GSH)) quantities in maize plants (*Zea mays* L. cv. Hido) exposed to boron toxicity (B) (8 mM $B(OH)_3$) and B + chitosan (0.01%, foliar (A) and seed soaking (B) applications) conditions in hydroponic culture have been studied comparatively. Boron toxicity significantly reduced growth parameters, chlorophyll, carotenoid, AsA, DHA and CAT activity while increased proline, MDA, oxidants ($O_2^{\cdot-}$ and H_2O_2), SOD, POD, APX and GR activities and GSH levels. B + Chitosan A and B applications significantly reversed the B toxicity-based inhibition in these parameters. It has been suggested that chitosan can be used as a reliable chemical for boron toxicity in maize, since chitosan applications (A and B) cause improvements in terms of all the parameters in the damage caused by B toxicty.

Key words: antioxidants, boron toxicity, chitosan, oxidative stress, maize

INTRODUCTION

Boron is an essential micro-nutrient element for the normal growth and development and the range of B concentration from its essentiality to toxicity is extremely narrow. Boron toxicity cause remarkable undesirable modifications on different growth processes such as metabolism, root cell division, photosynthesis, leaf chlorophyll content and some biochemical responses [Balal et al. 2017, Seth and Aery 2017, Rasha et al. 2019]. To halt losses triggered by this stress without damaging the ecosystem known to date to increase agricultural productivity there are some applications [Seth and Aery 2017].

Chitosan (β-1,4-linked D-glucosamine or 2-amino-2-deoxy-b-D-glucosamine) is a natural occurring carbohydrate biopolymer that derived from alkaline deacetylation of chitin in shrimp, crab shells, squid pens, lobster, cell walls of some fungi, nematode eggs and gut linings, insect exoskeletons and other crustaceans, and second most abundant polyaminosaccharide after cellulose [Balal et al. 2009, Mondal et al. 2012]. Chitosan being non-toxicity, hydrophilicity, polycationic, biodegradable, bioactivity, biocompatibility and adsorption properties finds numerous applications especially in the agricultural food and pharmaceutical industries, in removing heavy metals and dyes and in ideal natural support for enzyme immobilization.

Chitosan has an extensive scope of application such as from medicine to biotechnological applications. Chitosan as a biostimulant stimulate plant growth and yield, seed germination and abiotic stress tolerance including drought, salt and temperature stress and improve soil fertility by enhancing the mineral nutrient uptake and escalating nitrogen fixing nodes [Balal et al. 2017, Seth and Aery et al. 2017]. However, as with

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other plant hormone-like compounds, the useful effect of chitosan is generally depending on its concentration, application methods, environmental condition and developmental stage as well as the plant species [Balal et al. 2017].

Although there is some research about chitosan application on different stress conditions in some plants, there is no knowledge about how chitosan foliar spraying or seed soaking application would affect the growth of maize growing in Boron toxicity. The aim of this study was to determine the foliar spraying or seed soaking capacity and mechanisms involved in Chitosan-induced resistance of maize to B toxicity. Particular attention was focused to investigate the role of Chitosan in counteracting the adverse effects of B toxicity on maize plants at growth, chlorophyll, carotenoid, proline, MDA, $O_2^{\cdot-}$, H_2O_2, AsA, DHA and GSH quantities, and SOD, CAT, POD, APX, GR activities and its isoenzyme profiles. The results presented here demonstrate that the beneficial role of Chitosan on plants exposed to B toxicity appeared not to be related to retardation of B accumulation. We hope that this study will provide a basis for developing strategies to reduce the risks associated with B toxicity and maintaining sustainable maize production.

MATERIAL AND METHODS

Plant material and growth conditions. Chitosan A application (foliar spraying with chitosan solution). Before germination, seeds of maize (*Zea mays* L. cv. Hido) were surface sterilized in ethyl alcohol (95%) for 2 min and then transferred to sodium hypochloride activated with 1% Cl for 10 min, and washed with sterile dH_2O. After the sterilization, the seeds were incubated in petri dishes including wet filter paper for 72 h at 25°C in a germination cabinet. Uniform seedlings were selected and transferred into plate holes on hydroponic media containing a half-strength Hoagland nutrient solution. After 2 days of cultivation, the medium was changed to full-strength Hoagland solution. The seedlings were grown in a growth chamber (14 h photoperiod at 25 ±1°C, and 10 h dark at 20 ±1°C, relative humidity was approximately 70%) for 9 days. On the 9th day, the entire foliar region of the plants was foliar sprayed with chitosan (0.01%, dissolved in 0.5% acetic acid and adopted pH: 5.6 with 0.5 mM KOH during 3 days) or with only distilled water as a control (repeated three times at 2-h intervals (spraying chitosan onto leaves)). After 8 h chitosan application, then the plants exposed to B toxicity (8 mM, boric acid (H_3BO_3) Sigma-Aldrich, USA) for 3 days before harvesting. On the 12th day after the treatments were started, all the plants were harvested (the tissues were rinsed three times in distilled water after harvested) and analyzed (shown as Chitosan A application – C (A)) (Fig. 1).

Chitosan B application (seed soaking and soil application with chitosan solution). The imbibition of seeds were carried out into distilled water (control) and chitosan (0.01%, dissolved in 0.5% acetic acid and adopted pH: 5.6 with 0.5 mM KOH during 3 days) solution in plant growth cabinets in dark at 25°C for 6 h, the swelling period. Then swollen seeds (is a form of seed readiness in which seeds are pre-soaked before planting) were grown in control conditions for 9 days in a hydroponic culture system (each container was filled with 2.8 L of Hoagland and Arnon nutrient solution). On the 9th day, the plants exposed to B toxicity stress in the root medium (8 mM, boric acid (H_3BO_3)) for 3 days before harvesting. On the 12th day after the treatments were started, all the plants were harvested (the tissues were rinsed three times in distilled water after harvested) and analyzed (shown as Chitosan B application – C (B)) (Fig. 1).

The chitosan concentration, (0.01%, was optimal to enhance various morphological and growth attributes) in both applications was determined from a preliminary study comprising different levels of chitosan, foliar spraying and seed soaking on maize seedlings (data not shown) under moderate boron stress. Tween-20 as a surfactant was mixed with chitosan solutions prior to the spray. At the end of applications (12-d-old), plants were fractionated into roots and shoots. Immediately root and shoot samples were frozen in liquid nitrogen and stored at –80°C for some analysis.

Determination of chlorophyll, carotenoid and proline contents. Total chlorophyll and carotenoid contents (mg g^{-1} fresh mass) were assayed according to Witham et al. [1971]. Proline content was measured according to Bates et al. [1973].

Determination of superoxide anion production ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) content and lipid

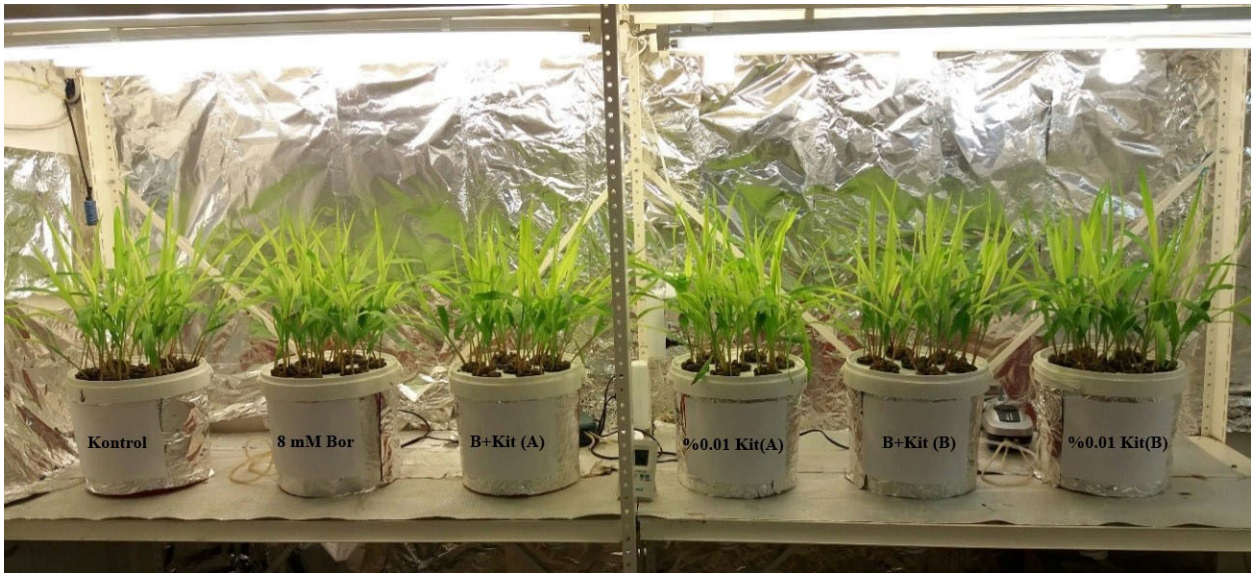

Fig. 1. Effects of B toxicity and Chitosan A and B applications on maize

peroxidation level (MDA). Superoxide level was measured according to Elstner and Heupel [1976]. Sodium nitrite was used as a standard solution to calculate the production rate of superoxide anion. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents were measured according to Loreto and Velikova [2001]. The MDA level (µmol g^{-1} fresh mass) was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and was expressed as µmol g^{-1} fresh mass.

Determination of antioxidant enzymes activities and isoenzyme profiles. For the enzyme assays (SOD, CAT, POD, APX and GR), leaf tissues (0.5 g) were homogenized in liquid nitrogen and 5 ml 10 mmol L^{-1} K–P buffer (pH 7.0) containing 4% (w/v) polyvinylpyrrolidone (PVPP) and 1 mmol L^{-1} disodium ethylenediamine tetraacetic acid (EDTA) was added. The homogenates were centrifuged at 12.000xg and 4°C for 15 min, and the supernatant was used to determine enzymes activities. SOD activity was measured according to Elstner and Heupel [1976]. SOD-PAGE was determined according to Beuchamp and Fridovich [1971]. CAT activity was measured according to Gong et al. [2001]. CAT-PAGE was determined according to Woodbury [1971]. POD activity was determined according to Yee et al. (2002). POD-PAGE was determined according to Liu [1973]. APX activity was determined according to Nakano and Asada [1981]. APX –PAGE was determined according to Mittler and Zilinskas [1993]. GR activity was determined according to Foyer ve Halliwell [1976]. GR –PAGE was determined according to Laemmli [1970].

Non-enzymatic antioxidant activity, ascorbate and glutathione. Tissue samples (0.2 g) were powdered in liquid nitrogen. Then 2 ml of 5 % (w/v) trichloro acetic acid (TCA) was added and homogenized. After centrifugation at 12,000xg for 10 min at 4°C, the supernatant was collected for determination of total ASA and GSH contents. Total ASA content (ASA + DHA) was estimated as described by Costa et al. [2005]. Total GSH, (nmol g^{-1} FW) was determined according to Wu et al. [2009]. Levels of GSH were estimated as difference between total GSH and GSSG.

Statistical analysis. The experiment was organized a completely random design with three replications. All data obtained were subjected to two-way analysis of variance (ANOVA) and significant differences between treatment means were determined by Duncan multiple range test using the SPSS 20.0. to compare means. Data are shown as means with three replicates and significance was determined at the 95% confidence (p 0.05) limits.

RESULTS AND DISCUSSION

Boron toxicity significantly reduced root-stem lengths compared with control in maize (Tab. 1). The results are consistent with previous results showing that B toxicity adversely affects plant productivity by disturbing photosynthetic pathways, membrane stability, photosynthetic pigments and production of reactive oxygen species [Seth and Aery 2017, Rasha et al. 2019]. Boron toxicity can cause retardation of elongation and cell division by binding to ATP and NADPH thereby disturbing their proper working in plant metabolism [Cervilla et al. 2009]. Chitosan A and B applications significantly alleviated boron toxicity induced inhibition in root-leaf lengths compared with individual boron toxicity in maize (Tab. 1). This positive effects of Chitosan applications A and B on plant growth occurred not only in the presence of stress but also in non-stressed plants because the highest values in root-stem lengths was recorded at individual Chitosan A and B applications. Smilar results reported in different plants that chitosan alleviated boron toxicity induced inhibitions in above parameter [Balal et al. 2017]. Effect of chitosan to plant growth promotion can be caused chitosan-induced enhancement in primary metabolic pathways such as carbon metabolism (chitosan treatment increased glyceraldehyde-3-phosphate dehydrogenase and phosphoglucomutase) photosynthesis, glycolysis and nitrogen metabolism [Chamnanmanoontham et al. 2015] or can be stimulate some signaling pathway related to plant hormones such as gibberellins and auxin [Safikhan et al. 2018].

B toxicity decreased chlorophyll a, b, total chlorophyll and carotenoid contents in the maize leaves (Tab. 1). Some researcher also reported that B toxicty also caused in alterations of photosynthetic pigment content and its relative proportion, e.g. chlorophyll *a*, *b* content and *a*/*b* ratio and carotenoid [Rasha et al. 2019]. All these effects are not related to a particular target of B toxicity at cellular level but are rather the observed responses of the ability of B to form complexes to molecules which are involved in different cellular processes. B toxicity causes the downregulation of photosystem biochemical components and inhibition of the electron transport rate. B toxicity also lowers the activity of carbon fixation enzymes [Chen et al. 2014]. Chitosan applications A and B significantly increased chlorophyll a, b and total chlorophyll and carotenoid contents in leaf compared with individual B toxicity (Tab. 1). Individual Chitosan A and B applications generally decreased chlorophyll a, b and total chlorophyll and carotenoid contents in the leaf compared with control in maize. Carotenoids could be involved in the protection against oxidative stress triggered by B toxicity. Zhang et al. [2011] reported that the chloroplast is recommend to be the fundamental organelle for chitosan action. As a result of chitosan application, numerous authors have been confirmed an increase in the chlorophyll content and upregu-

Table 1. Effects of Chitosan A and B applications on the growth parameters, chlorophyll a, b, total chlorophyll, carotenoid and proline contents in the root and leaf of 12 days old maize seedlings exposed to B toxicity

Applications	% inhibitions in growth		Chlorophyll (mg/g FW)		Total chlorophyll (mg/g FW)	Total carotenoid (mg/g FW)	Proline content (μmol g^{-1} FW)	
	root	leaf	Cl_a	Cl_b			root	leaf
Control			0.387 ±0^{b}	0.154 ±0^{a}	0.541 ±0^{a}	2.72 ±0.003^{a}	39.07 ±0.01^{a}	57.09 ±0.04^{b}
8 mM B	–18	–9.8	0.322 ±0^{f}	0.133 ±0^{e}	0.455 ±0^{f}	2.44 ±0.003^{d}	27.73 ±2.4^{b}	67.83 ±0.25^{a}
B + %0.01C (A)	26	6.7	0.352 ±0^{c}	0.146 ±0^{b}	0.498 ±0^{c}	2.63 ±0.006^{b}	34.74 ±2^{ab}	52.08 ±0.43^{c}
B + %0.01C (B)	13.7	3.6	0.331 ±0^{e}	0.136 ±0^{d}	0.467 ±0^{e}	2.44 ±0.029^{d}	31.88 ±3.1^{ab}	52.18 ±0.13^{c}
%0.01 C (A)	14.6	-2.1	0.392 ±0^{a}	0.144 ±0^{c}	0.536 ±0^{b}	2.74 ±0.016^{a}	35.44 ±3.3^{a}	52.82 ±0.49^{c}
%0.01 C (B)	5.1	3	0.345 ±0^{d}	0.145 ±0^{bc}	0.493 ±0^{d}	2.53 ±0.045^{c}	34.31 ±0.11^{b}	52.58 ±0.04^{c}

Data are the means ±SD of three independent replicates. Different small letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test at $p < 0.05$

lated of some proteins (oxygenevolving enhancer protein 1, chlorophyll A–B binding protein and ribulose bisphosphate carboxylase small chain) in plants [Chamnanmanoontham et al. 2015]. As a result of chitosan application, the increased chlorophyll content is also associated with an enhanced growth or increased net photosynthesis rate [Mondal et al. 2012], and may be caused by plants' enhanced uptake of nutrients (N, P, K, Ca and Mg) [Van et al. 2013]. Improving photosynthetic capacity can increase plant biomass, and chitosan contained about 8.7% N can promote the plant growth, development and their morphology [Farouk and Amany 2012]. Chitosan can protect membranes from the deleterious effect of B toxicity stress by induction enzyme activities or overexpression of some responsible genes in the photosynthesis, and can protect the photosynthetic apparatus [Xia et al. 2009]. Thus chitosan can reduce the effects of abiotic stress on plants.

B toxicity increased proline content in the leaf but decreased in the root compared with control in maize (Tab. 1). Increase in proline level and enhanced H_2O_2 accumulation has been considered as a common response of plants under B toxicity conditions [Balal et al. 2017, Seth and Aery 2017, Rasha et al. 2019]. Our result support that the photosynthetic pigments and proline are both synthesized from the same substrate. Therefore, proline accumulation leads to a decrease in photosynthetic pigments (Cl_a and $_b$) under B toxicity. Thus, the decline in photosynthetic pigments (Cl_a and $_b$) could be, at least in part, due to rise in proline accumulation [Balal et al. 2017]. Besides acting as an excellent osmolyte, proline acts as an inducer of metal chelators, antioxidative defense systems and a signaling molecule for resistance mechanisms activation [Hayat et al. 2012]. Proline is also known as the activator of the Krebs cycle reactions, thereby increasing the photosynthesis reactions rate and subsequent energy flow of plants. Proline accumulates in response to ROSs enhances the antioxidant capacity under stress conditions leading to decreased ROSs and proline content [Rejeb et al. 2014]. Chitosan A and B applications increased proline content in the root but decreased in the leaf compared with individual B toxicity in maize (Tab. 1). Individual Chitosan A and B applications decreased proline contents in the root and leaf compared with control in maize. Plants treated with Chitosan A and B will have less leaf/root proline concentration under B toxicity. However, Balal et al. [2017] reported that B toxicity importantly enhanced the proline content in cucumber (*Cucumis sativus* L), but chitosan applications increased these attributes in greater number. The effect of chitosan on reduction of B toxicity was also due at least partly to its stimulatory influence on proline accumulation. Proline (nitrogen-enriched) can utilize as endogenous source of nitrogen, to stabilize structure of protein and enzymes and photosynthesis apparatus and to protect cellular homeostasis under abiotic stress conditions by plants [Seth and Aery 2017]. So it may also be a possible reason for enhanced proline accumulation in response to chitosan in B toxicity.

The generation of ROS is one of the earliest responses of plants to different stress conditions [Rasha et al. 2019]. B toxicity importantly induced ROS (includes free radicals such as superoxide anion ($O_2^{\bullet-}$) as well as nonracial molecules like hydrogen peroxide (H_2O_2)) and MDA contents in the root and leaf compared with control in maize (Tab. 2). In accordance with the present results, some researchers reported that B toxicity may also cause increases in ROS production and lipid peroxidation [Balal et al. 2017, Rasha et al. 2019]. Under stressful conditions, ROS and free radicals are produced to enhance the activation of different resistance mechanisms. Accordingly, the cells employ different antioxidant systems to detoxify the ROS and free radicals. If the cells are able to produce enough amounts of antioxidants, they will combat the oxidative stress [Balal et al. 2017, Rasha et al. 2019]. Chitosan application A and B applications caused minimum rate of H_2O_2 and LPO than those of without chitosan. Positive effects of Chitosan A and B on these parameters occurred not only in the presence of B toxicity but also in non-stressed plants because individual Chitosan A and B applications importantly decreased $O_2^{\bullet-}$, H_2O_2 and MDA contents in the root and leaf compared with control (Tab. 2). Chitosan has been reported to reduce MDA accumulations under water, cadmium toxicity and under low temperatures [Sarabandi et al. 2019]. Chitosan A and B applications have reduced the $O_2^{\bullet-}$, H_2O_2 and MDA contents while increased the different antioxidant enzymes activities. It is suggesting that this compound play a role in the ROS quenching or inhibitor of ROS production [Balal

Table 2. Effects of Chitosan A and B applications on superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and MDA contents in the root and leaf of 12 days old maize seedlings exposed to B toxicity

Applications	$O_2^{\cdot-}$ (nmol.min g^{-1} FW)		H_2O_2 (µmol g^{-1} FW)		MDA (nmol ml^{-1} FW)	
	root	leaf	root	leaf	root	leaf
Control	4.05 ±0.001^{b}	13.53 ±0.001^{a}	5.13 ±0.004^{e}	9.57 ±0.005^{b}	0.15 ±0.00^{b}	0.52 ±0.00^{c}
8 mM B	4.66 ±0.002^{a}	14.08 ±0.001^{a}	8.16 ±0.060^{a}	11.51 ±0.013^{a}	0.19 ±0.002^{a}	0.59 ±0.002^{e}
B + %0.01C (A)	3.67 ±0.002^{e}	11.87 ±1.33^{b}	6.02 ±0.003^{c}	6.71 ±0.060^{d}	0.14 ±0.001^{c}	0.56 ±0.002^{d}
B + %0.01C (B)	1.59 ±0.001^{f}	12.85 ±0.009^{a}	6.74 ±0.039^{b}	7.68 ±0.049^{c}	0.10 ±0.001^{f}	0.50 ±0.002^{b}
%0.01 C (A)	3.86 ±0.002^{c}	11.96 ±0.002^{b}	4.78 ±0.016^{f}	5.75 ±0.076^{e}	0.13 ±0.001^{d}	0.43 ±0.002^{a}
%0.01 C (B)	3.85 ±0.002^{d}	11.90 ±0.002^{b}	5.40 ±0.083^{d}	9.57 ±0.052^{b}	0.11 ±0.00^{e}	0.50 ±0.002^{b}

Data are the means ±SD of three independent replicates. Different small *letters* indicate significant differences at $P < 0.05$ according to Duncan's multiple range test at $p < 0.05$

Table 3. Effects of Chitosan A and B applications on total AsA, DHA and glutathione (GSH) contents in the root and leaf of 12 days old maize seedlings exposed to Boron toxicity

Applications	Total AsA (nmol g^{-1} FW)		DHA (nmol g^{-1} FW)		Total GSH (nmol g^{-1} FW)	
	root	leaf	root	leaf	root	leaf
Control	152 ±1.45b	184 ±0.88e	113 ±0.33c	103 ±0.88e	263 ±0.58c	253 ±0.26f
8 mM B	85 ±1.15d	211 ±0.88c	62 ±0.66e	119 ±0.88d	277 ±0.64b	578 ±0.38b
B + %0.01C (A)	116 ±1.15c	144 ±0.88f	91 ±0.33d	91 ±0.0f	329 ±0.71a	603 ±0.37a
B + %0.01C (B)	151 ±1.20b	332 ±1.73a	116 ±0.57b	198 ±0.33a	219 ±1.24d	285 ±0.54e
%0.01 C (A)	162 ±0.88a	197 ±0.88d	127 ±0.57a	127 ±0.33c	276 ±0.85b	539 ±0.17c
%0.01 C (B)	160 ±1.15a	280 ±1.45b	117 ±0.33b	168 ±0.88b	183 ±0.78e	414 ±0.49d

Data are the means ±SD of three independent replicates. Different small letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test at $p < 0.05$

et al. 2009] and is able to retain cell integrity by enhancing the antioxidant capacity of the cells. Chitosan treated plants showed better $O_2^{\bullet-}$ scavenging ability to protect the plant from oxidative damage related B toxicity. Chitosan as related with its structure (its abundant active hydroxyl and amino groups) is an excelleet scavenger of hydroxyl radicals, H_2O_2 and anion superoxide that is chitosan and SOD have similar abilities to scavenge superoxide anion ($O_2^{\cdot-}$) [Sun et al. 2004]. The improved growth of B-stressed wheat plants might be connected with the potential role of Chitosan in stabilizing membranes. The role of applied Chitosan under B toxicity in plants is not yet clear and needs further investigations.

Glutathione and ascorbate are superoxide scavenging antioxidants. Other antioxidants, that is, phenolics and flavonoids, can also enhance the ability of plants to cope with ROS produced under boron toxicity [Lee and Scagel 2009]. In the latest reports, there has been also understand a close correlation between B and total AsA [Brown et al. 2002]. In this study, B toxicity decreased total AsA (redox metabolite) and DHA contents in the root but increased in the leaf (Tab. 3). Chitosan A and B applications generally increased in both root and leaf exposed to B toxicity except for C (A) application in the leaf. Individual Chitosan A and B applications increased total AsA and DHA contents in the root and leaf compared to control (Tab. 3). B toxicity increased total GSH contents in the root and leaf of maize. Our results are in according with Wang et al. [2011] who also showed an increase in total GSH level in pear leaves under B toxicity. Chitosan A ap-

Table 4. Effects of Chitosan A and B applications on SOD, CAT, POD, APX and GR activities in the root and leaf of 12 days old maize seedlings exposed to B toxicity

Applications	SOD (U mg^{-1} protein FW)		CAT (μmol. min^{-1} mg^{-1} protein FW)		POD (U mg^{-1} protein FW)		APX (U mg^{-1} protein FW)		GR (U mg^{-1} protein FW)	
	root	leaf	root	leaf	root	leaf	root	leaf	root	leaf
Control	6.85 ±0.04^{d}	2.93 ±0.010^{d}	2.90 ±0.018^{b}	2.47 ±0.02^{d}	2604 ±5.45^{d}	823 ±6.7^{c}	8.49 ±0.10^{e}	5.58 ±0.05^{d}	7.61 ±0.12^{e}	72.03 ±0.05^{c}
8 mM B	8.20 ±0.14^{a}	3.53 ±0.008^{a}	1.29 ±0.015^{d}	1.44 ±0.02^{a}	3397 ±8.25^{a}	1110 ±11.5^{b}	33.66 ±0.03^{a}	9.22 ±0.05^{a}	12.70 ±0.13^{b}	72.42 ±0.07^{c}
B + %0.01C (A)	7.13 ±0.01^{c}	3.42 ±0.008^{b}	1.49 ±0.010^{c}	3.39 ±0.25^{b}	3104 ±31.34^{b}	1205 ±10.6^{a}	13.46 ±0.15^{c}	8.34 ±0.04^{b}	10.98 ±0.18^{c}	66.63 ±0.29^{e}
B + %0.01C (B)	7.39 ±0.01^{b}	2.91 ±0.010^{d}	2.85 ±0.018^{b}	0.68 ±0.04^{d}	2673 ±13.8^{cd}	825 ±14.3^{c}	28.68 ±0.06^{b}	7.68 ±0.02^{c}	10.84 ±0.16^{c}	101.67 ±0.29^{b}
%0.01 C (A)	6.88 ±0.03^{d}	3.15 ±0.002^{c}	2.10 ±0.004^{b}	2.12 ±0.01^{c}	2232 ±20.6^{e}	661 ±8.1^{e}	7.26 ±0.03^{f}	4.61 ±0.25^{e}	9.71 ±0.30^{d}	70.91 ±0.17^{d}
%0.01 C (B)	7.28 ±0.08^{bc}	2.83 ±0.009^{e}	5.33 ±0.016^{a}	1.54 ±0.02^{e}	2656 ±11.2^{c}	781 ±8.6^{d}	10.32 ±0.04^{d}	3.65 ±0.01^{f}	13.40 ±0.25^{a}	109.32 ±0.26^{a}

Data are the means ±SD of three independent replicates. Different small letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test at $p < 0.05$

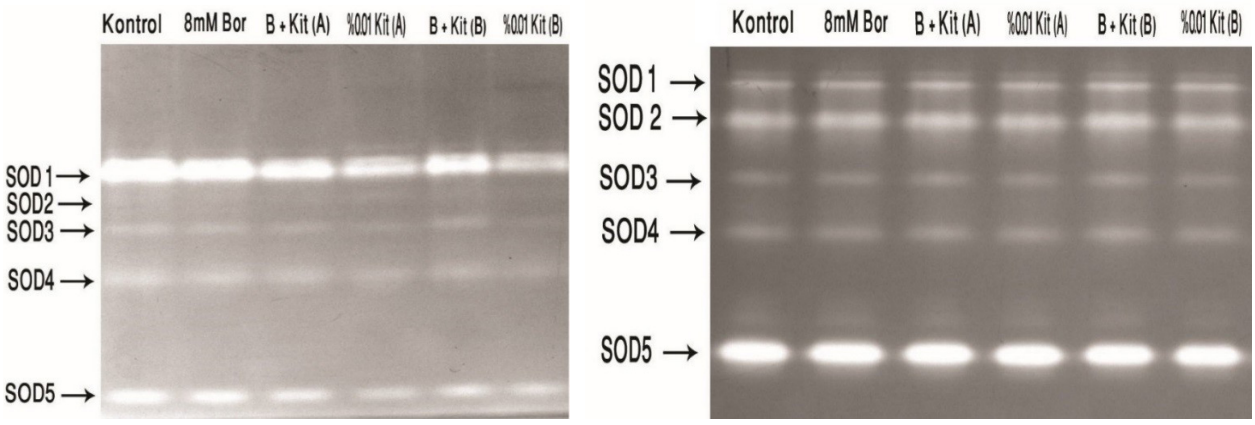

Fig. 2. Effects of Chitosan A and B applications on SOD isoenzyme profile in 12 days old maize root and leaf exposed to B toxicity

plication more increased total GSH content in the root and leaf but Chitosan B application decreased it in both tissues. Individual Chitosan A and B applications generally increased total GSH contents in both tissues. In this study, chitosan induced the AsA–GSH cycle and caused increases in the total AsA and GSH according to control. Increased antioxidant activity associated with GSH accumulation can protect many photosynthetic enzyme activities and is related with the capability of plants to withstand B toxicity-induced oxidative stress in B toxicity. Furthermore, less oxidative damage was reported in chitosan treated plants by increasing non-enzymatic (total AsA and GSH) compounds [Wang et al. 2011]. Finally, the stimulation of GSH

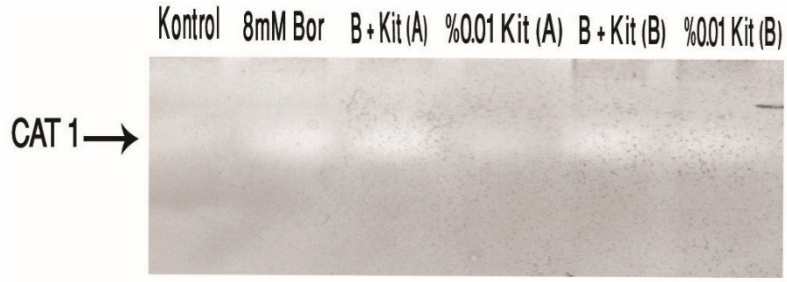

Fig. 3. Effects of Chitosan A and B applications on CAT isoenzyme profile in 12 days old maize leaf exposed to B toxicity

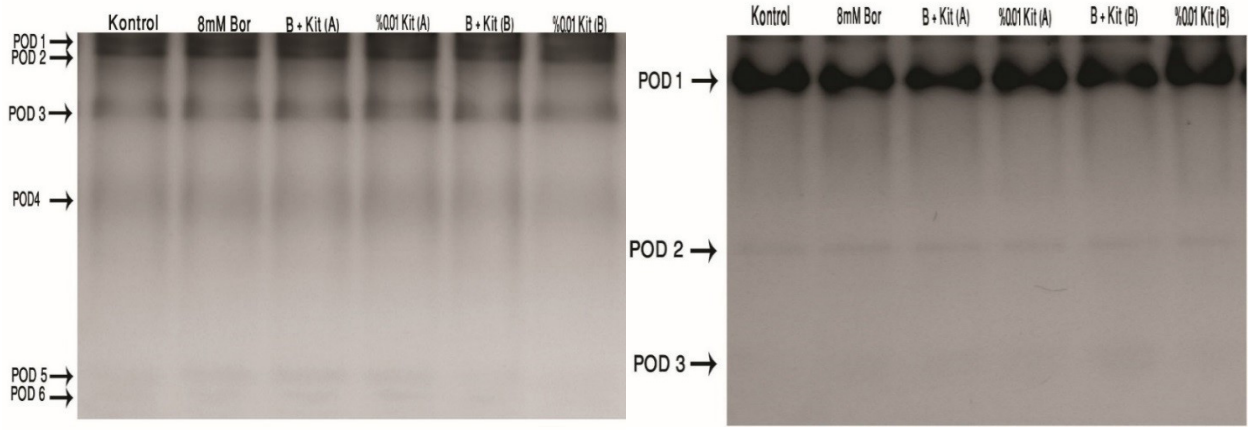

Fig. 4. Effects of Chitosan A and B applications on POD isoenzyme profile in 12 days old maize root and leaf exposed to B toxicity

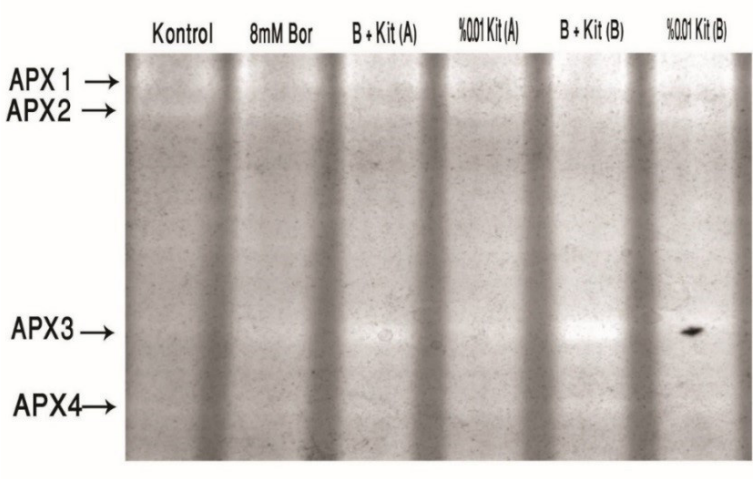

Fig. 5. Effects of Chitosan A and B applications on APX isoenzyme profile in 12 days old maize leaf exposed to B toxicity

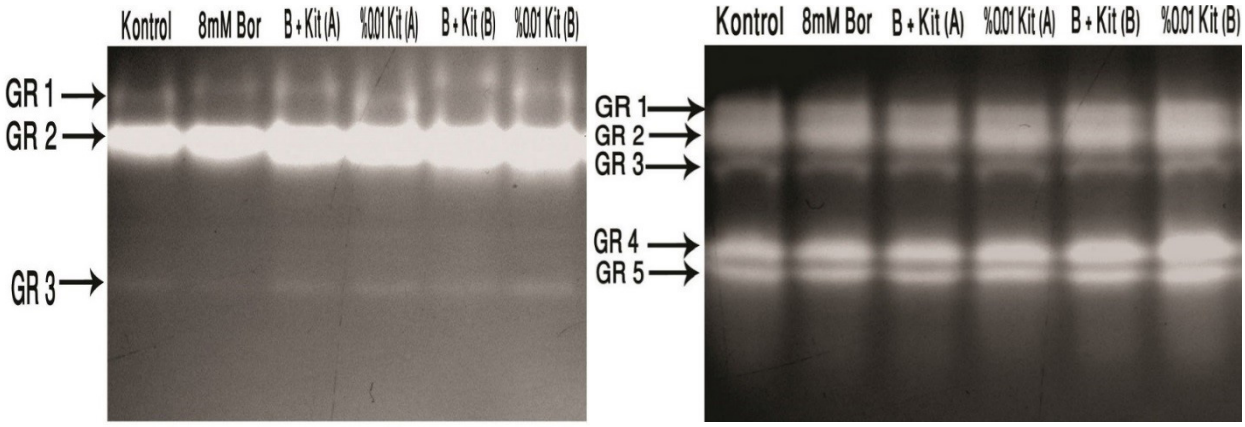

Fig. 6. Effects of Chitosan A and B applications on GR isoenzyme profile in 12 days old maize root and leaf exposed to B toxicity

synthesis like AsA as a most powerful ROS scavenger could present a protective function against oxidative stress by B toxicity.

In this study, B toxicity increased SOD, POD (by using guaiacol substrate), APX and GR activities but decreased CAT activity in both root and leaf in maize, and these results supported by isoenzyme profiles (Tab. 4 and Fig. 2–6). Responses of enzymatic antioxidants against to B toxicity change importantly depend on species and stresses. Our results support the previous results that they have found increases in antioxidant responses against to B toxicity [Balal et al. 2017]. These results show that maize has the capacity to adapt to B toxicity by promoting antioxidant defense system because these enzymes are considered as important tolerance mechanism in maintaining ROS levels by depressing or keeping ROS in normal and stress conditions. On the other hand, B + Chitosan A and B applications and individually chitosan A and B applications generally reduced SOD, POD, APX and GR activities but increased CAT activity in both root and leaf. Some resaechers also found that the enzyme activity and/or transcript level of CAT was induced by chitosan treatment in different stress conditons [Povero et al. 2011]. The increased expression of CAT due to treatment of chitosan might provide the required protection to the plants from the oxidative stress. In this study, SOD activity was inhibited by Chitosan A and B applications in the root and leaf with B toxicity. But $O_2^{\bullet-}$ content was decreased by Chitosan A and B applications in the root and leaf. In individually chitosan A and B applications as expected the lowest value of $O_2^{\bullet-}$ because of high SOD activities was determined. Chitosan can be improve plant defense by reducing ROS level both in generation stage via activation of scavenging them through activation of antioxidant system. This show that Chitosan can combine with intercellular $O_2^{\bullet-}$ by reducing oxygen tension that provides more protection against to B toxicity in maize. But other researcher generally found that Chitosan applications increased activity and/or up-regulated transcript level of SOD, POD, APX and GR in control and different stress conditions [Balal et al. 2017, Povero et al. 2011]. Our results don't support their results. However, it should be noted that plant response to chitosan might vary depending on the type of the used chitosan and plant species and developmental stages [Pichyangkura and Chadchawan 2015].

CONCLUSIONS

As a result, we showed that chitosan both tested lines (A and B) played in a key role in required protection to the plants from the oxidative stress; by increasing cell membrane stability (decline in MDA content) during B toxicity; by elevating the adverse reactions of ROS towards membranes and reduce the level of superoxide anion radicals and hydrogen peroxide; by increasing proline content and CAT activity. Our results showed that foliar spray application of chitosan

(B + C (A)) was more effective than its seed soaking and soil application with chitosan solution (B + C (B)) in alleviating the B-induced deleterious effects because it markedly minimized the B toxicity-induced reduction in above defined growth properties. It has been suggested that chitosan can be used as a reliable chemical for boron toxicity in maize, since chitosan applications (A and B) cause improvements in terms of all the parameters in the damage caused by boron toxicty.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHOR CONTRIBUTION STATEMENT

Dr. Yavuz Demir designed the experimental framework and performed paper writing. Dr. Sakineh Mohammadi Kohnehshahri performed all the experiments and the statistical analysis of data.

REFERENCES

Balal, R.M., Shahid, M.A., Javaid, M.M., Iqbal, Z., Liu, G.D., Zotarelli, L., Khan, N. (2017). Chitosan alleviates phytotoxicity caused by boron through augmented polyamine metabolism and antioxidant activities and reduced boron concentration in *Cucumis sativus* L. Acta Physiol. Plant, 39(1), 31.

Bates, L., Waldren, R., Teare, I. (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39, 205–207.

Beuchamp, C., Fridovich, I. (1971). Isozymes of superoxide dismutase from wheat germ. Biochim. Biophys. Acta, 317, 50–64.

Brown, P.H., Bellaloui, N., Wimmer, M.A., Bassil, E.S., Ruiz, J., Hu, H., Pfeffer, H., Dannel, F., Romheld, V. (2002). Boron in plant biology. Plant Biol., 4, 205–223.

Cervilla, L., Blasco, B., Ríos, J., Rosales, M., Rubio-Wilhelmi, M., Sánchez-Rodríguez, E., Romero, L., Ruiz, J. (2009). Response of nitrogen metabolism to boron toxicity in tomato plants. Plant Biol., 11(5), 671–677.

Chamnanmanoontham, N., Pongprayoon, W., Pichayangkura, R., Roytrakul, S., Chadchawan, S. (2015). Chitosan enhances rice seedling growth via gene expression network between nucleus and chloroplast. Plant Growth Regul., 75, 101–114.

Chen, M., Mishra, S., Heckathorn, S.A., Frantz, J.M., Krause, C. (2014). Proteomic analysis of *Arabidopsis thaliana* leaves in response to acute boron deficiency and toxicity reveals effects on photosynthesis, carbohydrate metabolism, and protein synthesis. J. Plant Physiol., 171, 235–242.

Costa, P., Neto, A., Bezerra, M. (2005). Antioxidantenzymatic system of two sorghum genotypes differing in salt tolerance. Braz. J. Plant Physiol., 17(4), 353–362.

Elstner, E.F., Heupel, A. (1976). Inhibition of nitrate formation from hydroxylammonium chloride: a simple assay for superoxide dismutase. Anal. Biochem., 70, 616–620.

Farouk, S., Amany, A.R. (2012). Improving growth and yield of cowpea by foliar application of chitosan under water stress. Egypt. J. Biol., 14, 14–16.

Foyer, C., Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta, 133, 21–25.

Gong, Y., Toivonen, P.M.A., Lau, O.L., Wiersma, P.A. (2001). Antioxidant system level in '*Braeburn*' apple in related to its browing disorder. Bot. Bull. Acad. Sin., 42, 259–264.

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A. (2012). Role of proline under changing environments: a review. Plant Signal. Behav., 7(11), 1456–1466.

Laemmli, D.K. (1970). Cleavage of structural proteins during in assembly of the heat of bacteriophage T4. Nature, 227, 680.

Lee, J., Scagel, C.F. (2009). Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. Food Chem., 115, 650–656.

Loreto, F., Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol., 127, 1781–1787.

Mittler, R., Zilinskas, B.A. (1993). Detection of ascorbate peroxidase activity in native gels by ınhibition of the ascorbate-dependent reduction of nitroblue tetrazolium. Anal. Biochem., 212(2), 540–546.

Mondal, M.M.A., Malek, M.A., Puteh, A.B., Ismail, M.R., Ashrafuzzaman, M., Naher, L. (2012). Effect of foliar application of chitosan on growth and yield in okra. Austral. J. Crop Sci., 6(5), 918–92.

Nakano, Y., Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol., 22, 867–880.

Pichyangkura, R., Chadchawan, S. (2015). Biostimulant activity of chitosan in horticulture. Sci. Hortic., (Amst.) 196, 49–65.

Povero, G., Loreti, E., Pucciariello, C., Santaniello, A., Di Tommaso, D., Di Tommaso, G., Kapetis, D., Zolezzi, F., Piaggesi, A., Perata, P. (2011). Transcript profiling of chitosan-treated Arabidopsis seedlings. J. Plant Res., 124, 619–629.

Rasha, M. El-Shazoly., Ashraf, A. Metwally., Afaf, M. Hamada. (2019). Salicylic acid or thiamin increases tolerance to boron toxicity stress in wheat. J. Plant Nutr., 42(7), 702–722.

Rejeb, K.B., Abdelly, C., Savouré, A. (2014). How reactive oxygen species and proline face stress together. Plant Physiol. Biochem., 80, 278–284.

Safikhan, S., Khoshbakht, K., Chaichi, M.R., Amini, A., Motesharezadeh, B. (2018). Role of chitosan on the growth, physiological parameters and enzymatic activity of milk thistle (*Silybum marianum* (L.) Gaertn.) in a pot experiment. J. Appl. Res. Med. Aromat. Plants, 10, 49–58.

Sarabandi, M., Farokhzad, A., Mandoulakani, B.A., Ghasemzadeh, R. (2019). Biochemical and gene expression responses of two Iranian grape cultivars to foliar application of methyl jasmonate under boron toxicity conditions. Sci. Hortic., 249, 355–363.

Seth, K., Aery, N.C. (2017). Boron induced changes in biochemical constituents, enzymatic activities, and growth performance of wheat. Acta Physiol. Plant., 39(11), 244.

Sun, T., Xie, W., Xu, P. (2004). Superoxide anion scavenging activity of graft chitosan derivatives. Carbohydr. Polym., 58(4), 379–382.

Wang, J.Z., Tao, S.T., Qi, K.J., Wu, J., Wu, H.Q., Zhang, S.L. (2011). Changes in photosynthetic properties and antioxidative system of pear leaves to boron toxicity, Afr. J. Biotechnol., 10(85), 19693–19700.

Witham, F.H., Blaydes, B.F., Devlin, R.M. (1971). Experiments in plant physiology. Van Nostrand Reinhold, New York, pp. 167–200.

Woodbury, W., Spencer, A., Stahman, M. (1971). An improved procedure using ferricyanide for detecting catalase isozymes. Anal. Biochem., 44(1), 301–305.

Wu, T., Hsu, Y., Lee, T. (2009). Effects of cadmium on the regulation of antioxidant enzyme activity, gene expression, and antioxidant defenses in the marine macroalga *Ulva fasciata*. Bot. Stud., 50, 25–34.

Xia, X.J., Huang, L.F., Zhou, Y.H., Mao, W.H., Shi, K., Wu, J.X., Asami, T., Chen, Z., Yu, J.Q. (2009). Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus*. Planta, 230, 1185–1196.

Van, S.N., Minh, H.D., Anh, D.N. (2013). Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house. Biocatal. Agric. Biotechnol., 2(4), 289–294.

Yee, Y., Tam, N.F.Y., Wong, Y.S., Lu, C.Y. (2002). Growth and physiological responses of two mangrove species (*Bruguira gymnorrhiza* and *Kandelia candel*) to waterlogging. Environ. Exp. Bot., 1–13.

Zhang, H., Zhao, X., Yang, J., Yin, H., Wang, W., Lu, H., Du, Y. (2011). Nitric oxide production and its functional link with OIPK in tobacco defense response elicited by chitooligosaccharide. Plant Cell Rep., 30, 1153–1162.