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BRIEF COMMUNICATION

Silicon increases boron tolerance and reduces oxidative damage of wheat grown in soil with excess boron

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Abstract

The effect of silicon on the growth, boron concentrations, malondialdehyde (MDA) content, lipoxygenase (LOX) activity, proline (PRO) and H_2O_2 accumulation, and the activities of major antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX)] and non-enzymatic antioxidants (AA) of wheat grown in soil originally with toxic B concentrations were investigated. Applied of 5.0 and 10.0 mM Si to the B toxic soil significantly increased Si concentration of the wheat and counteracted the deleterious effects of B on shoot growth. The contents of PRO, H_2O_2, MDA, and LOX activity of wheat grown in B toxic soil were significantly reduced by Si treatments. Compared with control plants, the activities of SOD, CAT, APX and content of AA were decreased by applied Si. Based on the present work, it can be concluded that Si alleviates B toxicity of wheat by preventing oxidative membrane damage and also translocation of B from root to shoot and/or soil to plant.

Additional key words: antioxidant enzymes, B toxicity, lipid peroxidation, lipoxygenase, proline, *Triticum aestivum*.

Boron is often found in high concentrations in association with saline-sodic soils. If such levels of boron are accompanied by excessive salinity, the consequences can be drastic for plants (Alpaslan and Gunes 2001, Ismail 2003). While of lesser prevalence than B deficient soils, B-rich soils are of great significance since its toxicity decreases plant growth and crop yields (Papadakis *et al*. 2004). Although of considerable agronomic importance, our understanding of B toxicity mechanisms in sodic soils is still not completely understood.

Under abiotic stress conditions reactive oxygen species (ROS) are commonly generated and accumulated (Mittler 2002) and cause oxidative damage to lipids and proteins and eventually lead to cell death (Molassiotis *et al*. 2006a). Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids, has been utilized as a suitable biomarker for lipid peroxidation (Mittler 2002). Lipid peroxidation can also be initiated enzymatically through a sequential action of lipoxygenases (LOX; EC 1.13.11.12), a ubiquitous plant enzyme that incorporates molecular oxygen into polyunsaturated fatty acids, to form lipid hydroperoxides (Axelrod *et al*. 1981). The antioxidant defense system in the plant cell includes both enzymatic such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11) and non-enzymatic antioxidants such as ascorbate, glutathione and α-tocopherol. As a major scavenger, SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen. However, H_2O_2 is also toxic to cells and has to be further detoxified by CAT and/or peroxidase (POD; EC 1.11.1.7) to water and oxygen (Zhu *et al*. 2004). In the ascorbate-glutathione cycle, APX reduces H_2O_2 using ascorbate as an electron donor. Altered activities of these antioxidant enzymes and antioxidants commonly have been reported, and used frequently as indicators of oxidative stress in plants (Mittler 2002). Under stress conditions, plants besides producing antioxidants also accumulate compatible solutes in the cytosol, such as proline that originally were thought to function as osmotic buffers. However, apart from osmotic adjustment it seems to play a role in maintaining the natural state of macromolecules probably by scavenging ROS (Xiong and Zhu 2002).

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Abbreviations: AA - non-enzymatic antioxidants; APX - ascorbate peroxidase; CAT - catalase; LOX - lipoxygenase; MDA - malondialdehyde; NBT - nitroblue tetrazolium; PRO - proline; ROS - reactive oxygen species; SOD - superoxide dismutase.

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Silicon is the second most prevalent element in the soil. Although abundant, silicon is never found in a plant available form and is always combined with other elements, usually forming oxides or silicates. Silicon is absorbed by plants in the form of uncharged silicic acid, $Si(OH)_4$, and is ultimately irreversibly precipitated throughout the plant as amorphous silica (Ranganathan *et al.* 2006). The importance of silicon has recently been recognized and the beneficial effects of Si in enhancing the tolerance of plants to biotic and abiotic stresses in several crops have been described (Epstein 1999, Ma 2004). Silicon effect on salt tolerance of barley, tomato and cucumber (Liang *et al*. 2003, Al-Aghabary *et al*. 2004, Zhu *et al*. 2004) have been related to antioxidant enzyme activity. To our knowledge, there is currently no information available about the possible beneficial effects of Si on B tolerance, the antioxidative system and stress markers in the performance of wheat grown in B toxic soils. In the present study, we used original B toxic soil in order to find out the effects of Si in real problematic growing conditions. The aim of the present work was to investigate the impact of Si on the growth, B uptake, lipid peroxidation, activity of LOX, PRO and H_2O_2 contents, the activities of major antioxidant enzymes (SOD, CAT and APX) and non-enzymatic antioxidants (AA) of wheat plants grown in B toxic conditions.

Wheat (*Triticum aestivum* L. cv. Bezostaja) plants were grown from November 02, 2005 to January 06, 2006 in a naturally lighted greenhouse at Faculty of Agriculture, Ankara University (39°57'44"N, 32°51'47"E). Experimental soil, typic natrargids, was collected from the plough layer (0 - 30 cm) of Akgol depression of the Great Konya Basin (Central Anatolia). Some characteristics of the soil were as follows: water retention capacity at 0.03 and 1.5 MPa 317 and 221 g kg^{-1}, respectively, texture clay, $CaCO_3$ 460.8 g kg^{-1}, pH (1:2.5 water) 8.48, EC 7.76 dS m^{-1}, organic matter 9.0 g kg^{-1}, total N 0.5 g kg^{-1}. Citric acid-extractable Si was 6.4 mg kg^{-1} and NH_4OAc-extractable K, Ca, Mg and Na were as follows (cmol kg^{-1}); 1.70, 10.4, 3.60 and 14.3, respectively. The $NaHCO_3$-available P was 7.75 mg kg^{-1}, and DTPA-extractable Zn, Fe and Mn were as follows (mg kg^{-1}); 0.95, 4.19 and 6.51, respectively. NaOAc-extractable B was 18.28 mg kg^{-1}. PVC pots were filled with 2 kg of air-dried soil. Treatments, with four replicates, consisted of control, 2.5, 5.0, and 10.0 mM Si applied as $Na_2Si_3O_7$ before sowing. For the basal fertilization, 100 mg N kg^{-1} soil from NH_4NO_3 and 50 and 62.5 mg P and K kg^{-1} soil from KH_2PO_4 were applied. Wheat seeds were sown at the rate of 10 seeds to each pot. and thinned to 6 plants per pot. During the experiment, soil was kept at approximately 60 % of the field capacity by watering with tap water.

All the measurements on fresh matter were done in the last week of November, 2005. At the end of the experiment, plants were harvested. After weighing (fresh mass), the shoots were washed once with tap water and twice in deionized water, dried in an air-forced oven at 60 °C until constant mass was reached (dry mass), and then ground (40 mesh sieve) for B and Si analyses.

All the enzymatic measurements were carried out at 0 - 4 °C. Fresh shoot samples (0.5 g) homogenized in a *Diax 900* homogenizer (*Heidolph*, Germany) in 5 cm^3 100 mM potassium phosphate buffer (pH 7.6) containing 1 mM EDTA-Na_2 and 0.5 mM ascorbate. The homogenized samples were centrifuged at 10 000 *g* for 5 min. The supernatant was used as crude enzyme extract. All activity measurements were made at 20 °C using a *UV/VIS 1201* spectrophotometer (*Shimadzu*, Japan). SOD activity was assayed by nitroblue tetrazolium (NBT) method (Gong *et al*. 2005). APX activity was determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 1 min in 2 cm^3 of a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA-Na_2, 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 0.05 cm^3 of crude enzyme extract at 25 °C (Nakano and Asada 1981). CAT activity was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2 (Cakmak *et al*. 1993). LOX activity was measured by initiating the reaction with the addition of 0.2 cm^3 enzyme extract in a 4 cm^3 of reaction mixture containing 50 mM sodium phosphate buffer (pH 6.5) and 0.4 mM linoleic acid (Axelrod *et al.* 1981).

Lipid peroxidation in the shoot tissue was determined by the thiobarbituric acid (TBA) test which determines MDA as an end product of lipid peroxidation (Hodges *et al.* 1999). The H_2O_2 content of the shoots was colorimetrically measured as described by Mukherjee and Choudhuri (1983). Free proline (PRO) content was extracted from 0.5 g of fresh shoot samples in 3 % (m/v) aqueous sulphosalicylic acid and estimated by ninhydrin reagent (Bates *et al.* 1973). Total non-enzymatic antioxidant activity (AA) assay was based on the reduction of Mo(VI) to Mo(V) and subsequent formation of a green phosphate/Mo(V) complex at acidic pH (Prieto *et al*. 1999).

Boron was determined colorimetrically at 420 nm by the azomethine-H method of Wolf (1971) and Si in the shoot tissues was determined by the blue silicon-molybdous procedure as described by Van der Vorm (1987).

The experiment was set up in a completely randomized design and each treatment contained four replicate pots. Analysis of variance was performed on the data, and significant differences among treatment means were calculated by Duncan's multiple range test ($P < 0.05$).

In the present study, the effects of Si on the growth, some physiological and enzymatic parameters symptomatic for oxidative stress and on the alleviation of B toxicity in wheat plants were assessed. Shoot dry mass was significantly lower when it was grown without supplemental Si. Growth reduction under combined saline and B toxic conditions was documented in tomato and cucumber (Alpaslan and Gunes 2001) and maize and sorghum (Ismail 2003). The fresh mass of wheat was not affected, but the dry mass was significantly increased by Si treatments (Table 1). Applied Si at 2.5, 5.0 and

Table 1. Shoot fresh and dry masses, Si, B, PRO, H_2O_2 and MDA contents, and activities of LOX, SOD, CAT, APX and non-enzymatic antioxidants (AA) in wheat plants grown in B toxic soil in the presence or absence of 2.5, 5.0 and 10.0 mM Si. The values are means of 4 replicates ± SE. Different letter in each row represents significant differences at $P < 0.05$ level, based on Duncan's multiple range test; *F-values*: ns - non-significant; ** - $P < 0.01$.

Parameters	Si [mM] 0	2.5	5.0	10.0	*F values*
Fresh mass [g plant^{-1}]	0.47 ± 0.03	0.53 ± 0.04	0.59 ± 0.04	0.55 ± 0.03	1.90^{ns}
Dry mass [g plant^{-1}]	0.09 ± 0.01b	0.12 ± 0.01a	0.13 ± 0.01a	0.12 ± 0.01a	6.79**
Si [g kg^{-1}]	5.00 ± 0.01b	5.10 ± 0.01b	6.51 ± 0.01a	7.43 ± 0.01a	9.65**
B [mg kg^{-1}]	505.0 ± 27.9a	499.0 ± 28.7a	411.0 ± 43.4b	285.0 ± 19.4b	10.88**
PRO [µmol g^{-1}(f.m.)]	0.44 ± 0.05a	0.23 ± 0.01b	0.26 ± 0.01b	0.31 ± 0.04b	6.30**
H_2O_2 [µmol g^{-1}(f.m.)]	49.22 ± 5.78a	22.86 ± 7.59b	18.44 ± 6.31b	18.44 ± 0.92b	7.29**
MDA [nmol g^{-1}(f.m.)]	17.98 ± 0.46a	13.45 ± 1.50bc	10.94 ± 1.31c	14.45 ± 0.67b	7.39**
LOX [mmol g^{-1}(d.m.)]	2.05 ± 0.17a	1.51 ± 0.08b	1.37 ± 0.18b	1.19 ± 0.11b	7.12**
SOD [Unit mg^{-1}(d.m)]	0.41 ± 0.01a	0.39 ± 0.02a	0.37 ± 0.01a	0.25 ± 0.01b	27.82**
CAT [mmol g^{-1}(d.m.) min^{-1}]	0.86 ± 0.01a	0.71 ± 0.08ab	0.52 ± 0.04c	0.86 ± 0.04bc	7.96**
APX [mmol g^{-1}(d.m.) min^{-1}]	12.32 ± 1.32a	6.97 ± 0.16c	9.51 ± 0.32b	8.74 ± 0.39bc	9.78**
AA [µmol g^{-1}(d.m.)]	83.75 ± 1.90a	66.11 ± 1.01c	71.70 ± 1.87b	65.96 ±1.28c	28.65**

10.0 mM improved the shoot dry mass of wheat grown in B toxic soil. There are no reports currently dealing with the effect of Si on B toxicity. However, Si has been shown to give yield increases under salt stress in tomato (Al-Aghabary *et al*. 2004, Romero-Aranda *et al*. 2006), cucumber (Zhu *et al*. 2004), and under drought stress in wheat (Gong *et al.* 2005).

The higher rates of Si application (5.0 and 10.0 mM) increased Si concentration of wheat plants. Romero-Aranda *et al.* (2006) also reported increases in Si concentration in the shoot of tomato in the presence of 2.5 mM Si. Application of Si at the rates of 5.0 and 10 mM significantly decreased the B concentrations in the shoot tissues of wheat. This decrease in B concentrations could possibly be because of the formation of boron-silicate complexes in the soil, and/or within the plant leading to lower B availability. The action of Si in reducing Na uptake in wheat (Ahmad *et al.* 1992) and rice genotypes (Yeo *et al*. 1999) has been previously reported, and the latter authors also reported salt-tolerant rice genotypes were least responsive to Si application. The possible another ameliorative effect of Si on the decreasing of B uptake could be related to Si being irreversibly precipitated as amorphous silica ($SiO_2.nH_2O$) in the cell walls and lumens as suggested by Richmond and Sussman (2003) and Ranganathan *et al.* (2006). This Si reinforcement of cell walls protects the plants from abiotic stresses and also reduces the translocation of salts to shoots (Epstein 1999).

Compared to control plants, the concentration of PRO was lower in Si treated plants and among the Si rates, the 2.5 mM Si was the most effective (Table 1). Increased proline content, together with enhanced H_2O_2 content is a common response of plants upon B stress treatments (Karabal *et al*. 2003), salinity (Bremont *et al.* 2006) and combined salinity and B toxicity (Alpaslan and Gunes 2001). In our experiment, compared with the Si supplied plants, H_2O_2 and PRO concentrations were significantly higher in the plants grown without Si supplement. Since the protective role of Si, with the decrease of B toxicity by supplemented Si. These results are consistent with the findings of Al-Aghabary *et al*. (2004) and Zhu *et al*. (2004) who showed that Si decreased salt-induced production of H_2O_2. The content of H_2O_2 was the highest in the control plants but decreased by Si treatments.

The content of MDA, produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damages. In the present study, lipid peroxidation (MDA content) and LOX activity were also significantly decreased by applied Si (Table 1). Decreased MDA content in barley by Si was also reported by Liang (1999). Excessive B- and salinity-mediated membrane damage was also previously reported in onion (Inal and Tarakcioglu 2001), tomato and cucumber (Alpaslan and Gunes 2001), and in sorghum and maize (Ismail 2003).

The wheat plants grown in B toxic soil without Si supplement exhibited the highest SOD, CAT and APX activities (Table 1). However, with the Si treatments, the activities of SOD, CAT and APX were significantly reduced. The non-enzymatic total antioxidant (AA) activity of wheat plants was also significantly reduced by 2.5, 5.0 and 10.0 mM Si (Table 1). The activity of SOD was almost paralleled with tissue H_2O_2 content. The results related with antioxidant responses are in agreement with the findings of Molassiotis *et al*. (2006a,b) who reported increases in the SOD, CAT, APX and AA activities under B toxicity in apple rootstocks. In addition to this, increased SOD and APX activities in barley under B-toxic conditions (Karabal *et al*. 2003) and in wheat under saline conditions (Sairam *et al*. 2005) has been reported.

In conclusion, the results of this study proved the role of Si in regulating B-toxicity stress response of wheat,

suggested that Si is involved in metabolic or physiological activities and indicated that Si could be used with the aim to improve plant growth under B toxic conditions. It was hoped that this study would provide a basis for developing strategies for reducing the risks of B toxicity and maintaining sustainable plant production.

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