ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 102, No. 2 (2015), p. 209–216
DOI 10.13080/z-a.2015.102.027

Oxidative stress responses in watermelon (*Citrullus lanatus*) as influenced by boron toxicity and drought

Mehmet HAMURCU[1], Tijen DEMIRAL[2], Erdoğan Eşref HAKKI[1], Önder TURKMEN[2], Sait GEZGIN[1], Richard W. BELL[3]

[1]Selçuk University
Campus Konya, Turkey

[2]Harran University
63300 Şanlıurfa, Turkey
E-mail: tijen.demiral@harran.edu.tr

[3]School of Environmental Science, Murdoch University
Perth, Western Australia

Abstract

This study aimed to investigate the effect of different boron (B) treatments on drought tolerance of watermelon plants. Drought tolerant *Citrullus lanatus* (Thunb.) Matsum. et Nakai genotype 'Kar 98' was grown in controlled greenhouse conditions hydroponically and exposed to drought stress by applying PEG 6000 (polyethylene glycol) in the presence of three boron dosages: 0.05, 0.25 and 1.25 mM. Growth parameters (fresh weight, dry weight and lengths of shoot and roots), leaf relative water content, boron accumulation, lipid peroxidation level and activities of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) enzymes were determined as well as the accumulation of hydrogen peroxide (H_2O_2) and hydroxyl (•OH)-scavenging activity were assayed. Increasing dosages of boron alone caused more severe growth reduction than combined with PEG 6000-induced drought stress. Induced drought stress caused less accumulation of boron in leaves and roots. B concentration of 1.25 mM caused lipid peroxidation in a reactive oxygen species-independent manner and drought stress-induced lipid peroxidation was alleviated by increasing B dosages. Induced glutathione reductase activity under the combination of 1.25 mM B and PEG 6000-induced drought stress seemed an important physiological response in 'Kar 98' plants against multiple stresses.

Key words: antioxidative activity, boron accumulation, hydrogen peroxide, lipid peroxidation, PEG 6000.

Introduction

Drought is one of the most important environmental factors affecting agricultural production and 26% of global arable lands (FAO, 2012). Drought stress may become more acute in future due to global warming and climate change mainly in Southern Europe. For example, in Turkey, climate is predicted to become drier and hotter by 2030. Hence, identification of drought-tolerant plant species and their tolerance mechanisms, as well as determination of the factors enhancing drought tolerance of plants, will ameliorate the effects of drought in the future.

Adequate boron (B) improves tolerance against abiotic stress conditions while deficient or toxic concentrations of boron cause oxidative stress itself. For example, both deficiency and toxicity of B supply have been shown to induce excessive production of ROS and thus peroxidation of lipid membranes in both leaves and roots of *Brassica* seedlings (Pandey, Archana, 2013). The activities of antioxidant enzymes are generally considered as a sign of stress tolerance of plant species against environmental stresses including drought. SOD constitutes the first line of antioxidative defense by converting superoxide to hydrogen peroxide (H_2O_2). In the presence of transition metals, H_2O_2 can interact with superoxide (O_2^-), which are toxic byproducts of oxidative metabolism, to generate highly reactive hydroxyl (•OH) radicals, which are believed to be mainly responsible for oxidative damage to lipid membranes and other biomolecules (Rahal et al., 2014). Boron accumulation in plant tissues has been shown to induce an oxidative load responsible for oxidative damage such as the damage evidenced on leaf lamina as necrotic and/or chlorotic areas through many studies. Consequently, the oxidative stress resulting from B deficiency/toxicity-induced an enhancement of the most powerful enzymatic antioxidants such as SOD, CAT, GR and APX (ascorbate peroxidase) in various plant species (Cervilla et al., 2007; Landi et al., 2013). B-deficient or toxic conditions have been shown to cause different trends in the activities of antioxidant enzymes by different studies. Increased accumulation of H_2O_2 in leaves and roots of *Brassica* was accompanied by enhanced activities of SOD, CAT, POX (peroxidase) and

PPO (polyphenol oxidase) under B deficiency and toxicity conditions (Pandey, Archana, 2013). In tomato leaves, GR and SOD activities were enhanced by high B levels whereas CAT activity was decreased (Keleş et al., 2011). In a similar study, GR and CAT activities in sunflower leaves were decreased by B concentrations higher than 40 μg g^{-1} (Keleş et al., 2011). Cervilla et al. (2007) also showed a remarkable increase in the accumulation levels of H_2O_2 and O_2^- in tomato leaves under high B levels and presented a clear correlation between leaf B accumulation and the amount of malondialdehyde (MDA) by-products. Growth reduction, which seems a general response to B toxicity, however, is not always alleviated by the induction in the antioxidative system (Keleş et al., 2011; Landi et al., 2013). For example, although high B concentrations increased GR and SOD activities in leaves of tomato, growth inhibition by boron toxicity still existed in these plants (Keleş et al., 2011). Nevertheless, some of the researchers have shown that increased growth under B toxicity in tolerant chickpea cultivar 'Gökçe' was correlated with induced activities of SOD, CAT and APX, as well as less accumulation of B than in sensitive cultivar 'Küsmen' (Ardic et al., 2009 b). Therefore, there are still conflicting results in the literature to establish the link between inducement of the antioxidant system and B toxicity tolerance. On the other hand, there is only limited information on the combined effects of various B concentrations and drought on watermelon.

Watermelon genotypes, which have a high economic value in Turkey, are known to sustain significant yield losses when exposed to drought conditions. Limited information is available about antioxidant responses of watermelon to drought; therefore, the aim of this research was to determine the contribution of different B dosages to tolerance mechanism of a drought-tolerant watermelon genotype 'Kar 98'. For this purpose, the role of antioxidants, lipid peroxidation level and reactive oxygen species accumulation in plant response were examined. To the best of our knowledge, the present report is the first available report that focuses on the effects of various B dosages in combination with PEG 6000-induced drought stress on antioxidative system of a drought-tolerant watermelon genotype.

Materials and methods

Drought-tolerant watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai genotype 'Kar 98' was used in this study. Plants were grown in a growth room with conditions of 45–55% humidity, 16 h light and 8 h dark photoperiod, 21 ± 1°C temperature and 14,000 lux/day light intensity. The seeds of watermelon were treated with 5% sodium hypochlorite for 10 min and thoroughly rinsed 3 times with sterile deionized water (dI-H_2O). After surface sterilization, seeds were imbibed for 2 h, put on humid filter papers in Petri plates, and then kept at 4°C overnight. Germinated seeds were floated on nylon net in 0.5 mM $CaCl_2$ solution at 25°C under dark and the seedlings were transferred to 1/5 Hoagland solution (pH 6.0) in continuously aerated growth room. Polyethylene glycol (PEG) of higher molecular weight has been employed quite frequently in physiological experiments to induce osmotic and drought stress (Türkan et al., 2005) as PEG molecules are not absorbed by seedlings. Therefore, in this study, we aimed to induce drought stress by exposing watermelon seedlings to 3.3% PEG 6000. Watermelon plants grown for 14 days hydroponically (until three-leaf stage) were exposed to drought stress for 10 days by adding 3.3% PEG 6000 within 1/5 Hoagland solution and to three different B dosages: 0.05 (control), 0.25 and 1.25 mM B. The group of plants not exposed to PEG 6000 treatment was used as control (the experimental chart is given in Fig. 1). Plants were harvested before (day 0) and 10 days after (day 10) PEG 6000 treatment and leaf samples were immediately frozen in liquid nitrogen, stored at −80°C until enzymatic analyses. The experiments were carried out at Selçuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition and repeated twice independently in 2012 and 2013 between May and July. The results of the experiments were similar and all data presented here are from representative experiments with similar results.

Growth parameters. Shoots and roots of watermelon plants were separated on harvest day (day 10) and their lengths, fresh weight (FW) and dry weight (DW) were determined after drying them at 70°C for 72 h in a draft oven.

Leaf relative water content (RWC). One cm diameter discs were cut from the interrib areas of six plants from fully opened fourth or fifth leaves from the shoot tip on harvest days 0 and 10. After determination of FW, the leaf disks were floated on distilled water in Petri dishes for 4 h under dark and then their turgid weights were weighed. The leaf disks were dried at 80°C for 24 h to determine DW. RWC was calculated according to Rouphael et al. (2008).

Boron measurement. Approximately 0.5 g of leaf and root samples harvested on day 10 were dried at 70°C and digested with concentrated HNO_3 in a microwave system CEM, Mars 5 (CEM Corp., USA). Boron concentration in the supernatant was analyzed by inductively coupled plasma atomic emission spectroscopy ICP-AES (Varian Vista AX, Australia).

Hydrogen peroxide (H_2O_2) accumulation level. H_2O_2 contents were estimated by using the method of Velikova et al. (2000). Leaf tissues (0.1 g) harvested on days 0 and 10 were homogenized with 1 mL of 0.1% (w/v) trichloroacetic acid (TCA) on an ice bath. Then homogenate was centrifuged at 12,000 × g for 15 min. The reaction mixture consisted of supernatant (0.5 mL), 0.5 mL potassium phosphate buffer (10 mM, pH 7.0) and 1 mL 1 M potassium iodide (KI), which was then vortexed, and the absorbance was measured at 390 nm, while 0.1% TCA was used as blank. The H_2O_2 content was determined from a standard curve, and the values were expressed as μmol g^{-1} fresh weight.

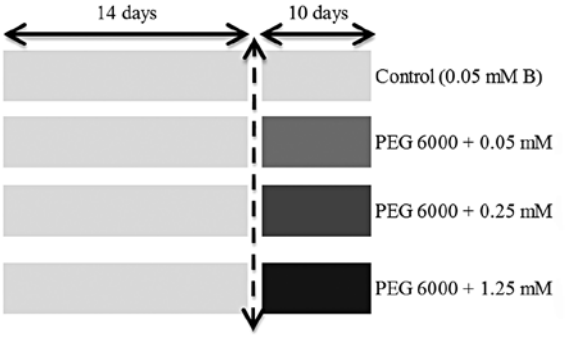


Figure 1. A chart showing the experimental design

Hydroxyl (•OH)-scavenging activity was determined according to 2-deoxyribose oxidation method (Chung et al., 1997). Absorbance at 520 nm was measured and inhibition rate of 2-deoxyribose oxidation by hydroxyl (•OH) radical was calculated as the percentage of inhibition according to the following formula:

$$\text{•OH-scavenging activity} = \left[\frac{(A0 - A1)}{A0}\right] \times 100,$$

where A0 is the absorbance of the control, A1 – the absorbance in the presence of leaf extracts.

Lipid peroxidation. The level of lipid peroxidation in leaves harvested on days 0 and 10 was determined in terms of malondialdehyde (MDA) content according to the method of Madhava Rao and Sresty (2000). The MDA concentration was calculated from the absorbance at 532 nm and measurements were corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Enzyme extractions and assays. Leaf samples (0.5 g) harvested on days 0 and 10 and having been stored at −80°C were homogenized with 50 mM sodium phosphate buffer (pH 7.8) containing 1 mM EDTA-Na_2 and 2% (w/v) polyvinylpolypyrrolidone (PVPP). For ascorbata peroxidase (APX) activity determination, 2 mM ascorbate was added into the homogenization buffer. All operations were performed at 4°C. Samples were centrifuged at 14,000 rpm for 40 minutes at 4°C, and supernatants were used for determination of protein content and enzyme activity assays. The total soluble protein contents of the enzyme extracts were determined according to Bradford (1976) using bovine serum albumin as standard. All spectrophotometric analyses were conducted on a UV-Visible 1600 spectrophotometer ("Shimadzu", Japan).

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by its ability to inhibit photochemical reduction of nitrobluetetrazolium (NBT) at 560 nm (Beauchamp, Fridovich, 1971). The assays were carried out at 25°C and the reaction mixture (3 mL) contained 0.033 mM NBT, 10 mM L-methionine, 0.66 mM EDTA-Na_2 and 0.0033 mM riboflavin in 0.05 mM sodium phosphate buffer (pH 7.8). Riboflavin was added last and the test tubes containing reaction mixture were incubated for 10 min under 300 µmol m^{-2} s^{-1} irradiance at 25°C. The reaction mixture with no enzyme developed the maximum colour due to maximum rate of reduction of NBT. Non-irradiated reaction mixture was used as the control as it did not develop colour. One unit of SOD activity was defined as the quantity of SOD required to cause a 50% inhibition of NBT, and the specific enzyme activity was expressed as units mg^{-1} protein.

Catalase (CAT, EC 1.11.1.6) activity was estimated according to Rao et al. (1997), which measures the initial rate of disappearance of H_2O_2 at 240 nm. The reaction mixture contained 0.05 M sodium-phosphate buffer (pH 7.0) with 0.1 mM EDTA (ethylenediaminetetraacetic acid) and 3% H_2O_2. The decrease in the absorption was followed for 3 min and 1 µmol H_2O_2 destroyed per min was defined as one unit of CAT.

Glutathione reductase (GR, EC 1.6.4.2) activity was measured according to Foyer and Halliwell (1976). The assay medium contained 0.025 mM sodium-phosphate buffer (pH 7.8), 0.5 mM GSSG (glutathione disulfide), 0.12 mM NADPH (nicotinamide adenine dinucleotide phosphate) Na_4 and 0.1 mL enzyme extracted in a final assay volume of 1 mL. NADPH oxidation was followed at 340 nm. The activity was calculated using the extinction coefficient of NADPH (6.2 mM^{-1} cm^{-1}). A unit of GR activity was defined as µmol ml^{-1} oxidized GSSG per min.

The specific enzyme activities were expressed as units mg^{-1} protein for all enzymes assayed.

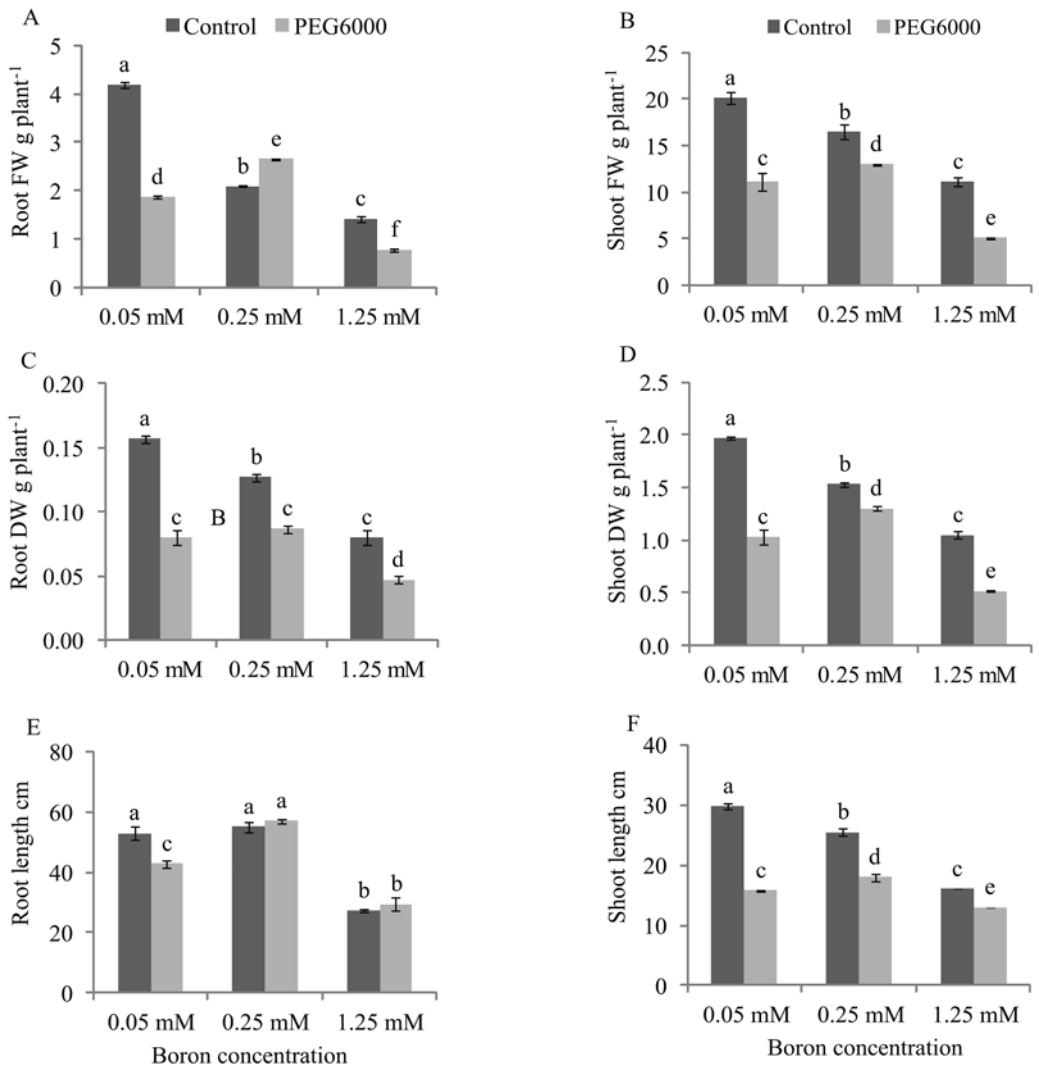
Statistical analysis. All analyses were done on a completely randomized design. All data obtained was subjected to one-way analyses of variance (ANOVA) and the mean differences were compared by a least significant difference (LSD) test. Each data point was the mean of six replicates (n = 6), and differences between mean values with $P < 0.01$ were considered significantly variant. In all the figures, the spread of values is shown as standard errors (SE) of the means.

Results and discussion

Growth parameters. Compared to control plants containing 0.05 mM B (Hoagland, Arnon, 1950), which is the adequate dose of B for most of the plants, 0.25 and 1.25 mM B treatments caused 50% and 66% decreases in root FW; 18% and 45% declines in shoot FW, respectively (Fig. 2 A and B). The dosages 0.25 and 1.25 mM B resulted in 19% and 49% decreases in root DW; 22% and 47% declines in shoot DW, respectively (Fig. 2 C and D). On the other hand, 0.25 and 1.25 mM B treatments caused 14% and 46% decreases in shoot lengths, respectively, while 1.25 mM B alone decreased root length by 49% (Fig. 2 E and F). Drought stress induced by PEG 6000 treatment reduced root FW, shoot FW, root DW, shoot DW, root length and shoot length by 55, 45, 49, 48, 19 and 47 %, respectively (Fig. 2). However, 0.25 mM B treatment seemed to alleviate the reductions in root and shoot FW, shoot DW, root and shoot lengths resulted from drought stress by causing 42, 17, 26, 33 and 14 % increases, respectively, in comparison to 0.05 mM B treatment (control) plants. Growth reduction resulting from B toxicity has also been reported in barley (Karabal et al., 2003), tomato (Cervilla et al., 2007; 2009) and wheat (Coskun et al., 2014).

Leaf relative water content (RWC). Leaf RWC of 'Kar 98' increased by 14% in 1.25 mM B treatment (Fig. 3 A), while it was not significantly affected by 0.25 mM B. Under PEG 6000-induced drought stress conditions, increasing B dosages did not affect leaf RWC content. Water restriction caused by drought stress seemed to prevent the increase in leaf RWC in 1.25 mM B-treated plants. Although we did not measure accumulation level of an osmoprotectant compound citrulline in 'Kar 98' plants in this study, lack of decrease in leaf RWC by PEG 6000 treatment regardless of B concentration applied might have been caused by increased accumulation of osmolytes such as citrulline (Yokota et al., 2002), which may help to lower the water potential and thus to retain cell water.

Boron accumulation. With 0.25 mM and 1.25 mM B supply, 'Kar 98' accumulated 2.2 and 5.9 times higher B in shoots, respectively, in comparison to 0.05 mM B (control group containing 74 mg kg^{-1} B in shoots) (Fig. 3 B). Furthermore, roots of 'Kar 98' accumulated 2.3 and 4.7 times higher B at 0.25 mM and



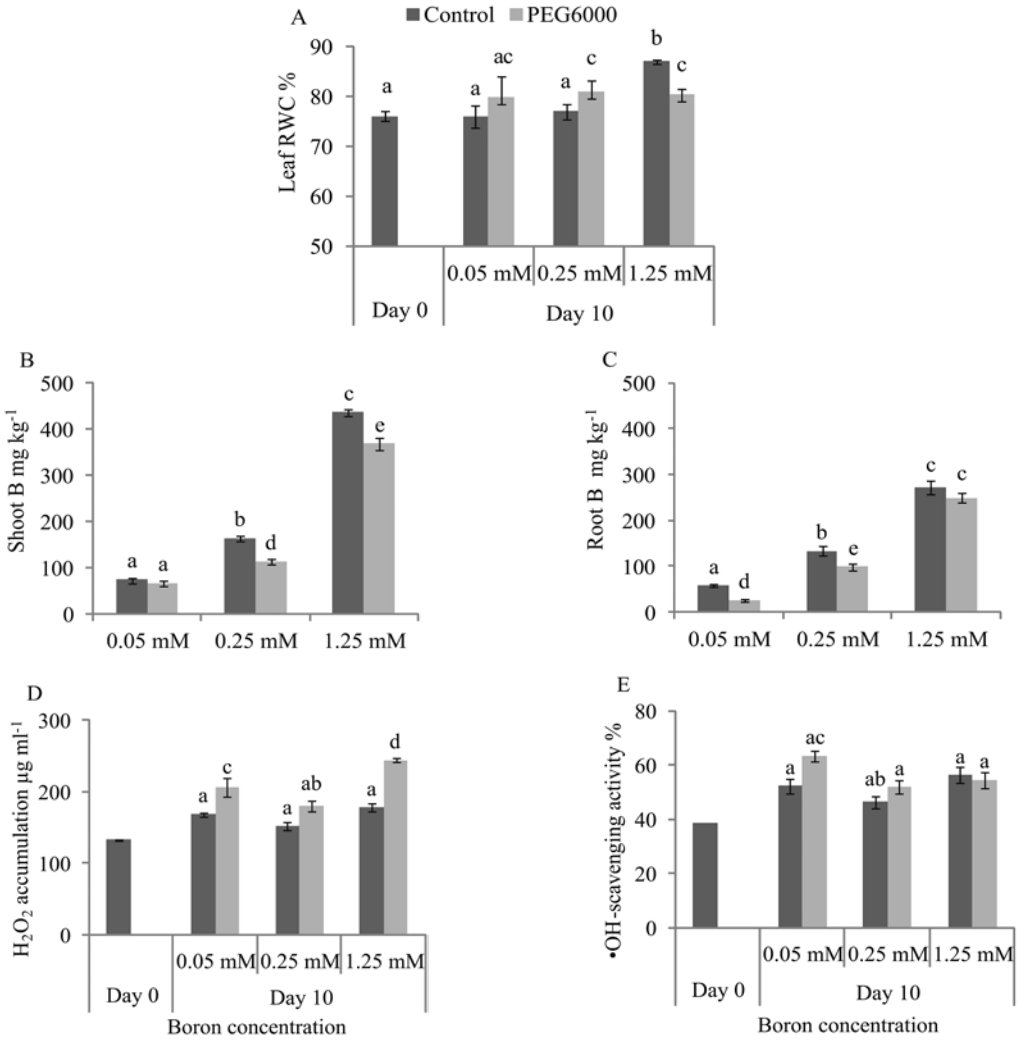
Notes. Values are the mean ± SE of two independent experiments (n ≥ 6). Means followed by the same letters are not significantly different at $P < 0.05$.

Figure 2. Effect of increasing boron dosages on growth parameters, including root fresh weight (FW) (A), shoot fresh weight (B), root dry weight (DW) (C), shoot dry weight (D), root length (E) and shoot length (F) of watermelon genotype 'Kar 98' after exposure to PEG 6000-induced drought stress for 10 days

1.25 mM B, respectively (Fig. 3 C). PEG 6000-induced drought stress caused 55% decrease in B accumulation in roots, while causing no change in B accumulation in shoots.

The 0.25 mM B treatment caused 31% and 26% decline in B accumulation in shoots and roots, respectively, in the presence of PEG 6000-induced drought stress. Although PEG 6000-induced drought stress did not affect the accumulation level of B in roots when 1.25 mM B was applied, a 16% decrease in B accumulation in shoots was observed (Fig. 3 B). Decreases in growth parameters caused by increasing B dosages were more prominent than those resulted from the combination of B toxicity and PEG 6000-induced drought stress. As PEG 6000-induced drought stress caused less accumulation of B in shoots and roots than in B treatment groups (Fig. 3 B and C), drought stress treatment seemed to have caused less growth reduction resulting from B toxicity. This was in support of correlation of intracellular B concentration with toxicity (Siddiqui et al., 2013). According to a model developed by Ben-Gal and Shani (2002), when a plant is exposed to B toxicity and salinity concurrently, yield of the plant is determined by the more harsh stress. Therefore, a dominant-stress-factor model of Liebig-Sperngel might validate decreased growth rate of watermelon plants with increasing B dosages in the present work (Ben-Gal, Shani, 2002). Cervilla et al. (2012) reported that a boron-sensitive tomato cultivar 'Josefina' showed a sharp decline in the biomass and leaf area on 10 days treatment of 0.5 mM B.

Reactive oxygen species. Neither 0.25 mM nor 1.25 mM B treatments altered H_2O_2 accumulation level in leaves of 'Kar 98' significantly (Fig. 3 D). However, PEG 6000-induced drought stress caused 18% increase in H_2O_2 accumulation level in control group including 0.05 mM B. When compared to control group containing 0.05, 0.25 and 1.25 mM B dosages caused 13% decrease and 18% increase in H_2O_2 accumulation under drought stress conditions, respectively. The •OH-scavenging activity in leaves of 'Kar 98' decreased 11% on 0.25 mM



Notes. Values are the mean ± SE of two independent experiments (n ≥ 6). Means followed by the same letters are not significantly different at $P < 0.05$.

Figure 3. Effect of increasing boron dosages on leaf relative water content (RWC) (A) on days 0 and 10, boron accumulation in shoots (B) and in roots (C) on day 10, hydrogen peroxide (H_2O_2) accumulation level (D) and hydroxyl (•OH)-scavenging activity (E) of watermelon genotype 'Kar 98' after exposure to PEG 6000-induced drought stress on days 0 and 10

B treatment while showing no significant change at 1.25 mM B (Fig. 3 E). PEG 6000-induced drought stress resulted in a 21% increase in •OH-scavenging activity in comparison to 0.05 mM B treatment (control group). Treatments of 0.25 mM and 1.25 mM B caused 18 and 14% decrements in •OH-scavenging activity under drought stress in comparison to control.

Boron toxicity has been reported to cause oxidative stress by promoting the accumulation of reactive oxygen species (ROS) (Pandey and Archana, 2013; Siddiqui et al., 2013). In the present study, however, H_2O_2 accumulation was not affected by increasing B dosages (Fig. 3 D) and 1.25 mM B alone enhanced peroxidation of lipid membranes in the absence of drought stress. Similarly, in a previous study on barley plants exposed to toxic B, Karabal et al. (2003) found no significant change in H_2O_2 levels but determined a dose-dependent increase in MDA content in shoots. Similarly, Cervilla et al. (2009) reported that high concentrations of B did not cause membrane damage in tomato roots. On the other hand, PEG 6000-induced drought stress in combination with B treatments promoted H_2O_2 accumulation more than control conditions in the present study. Lack of decrease in •OH-scavenging capacity in watermelon plants under both B toxicity and PEG 6000-induced drought stress also implied reduced level of highly reactive •OH radical formation through interaction of O_2^- with H_2O_2 through the Haber-Weiss reaction by means of enhanced SOD activity (Fig. 4 B).

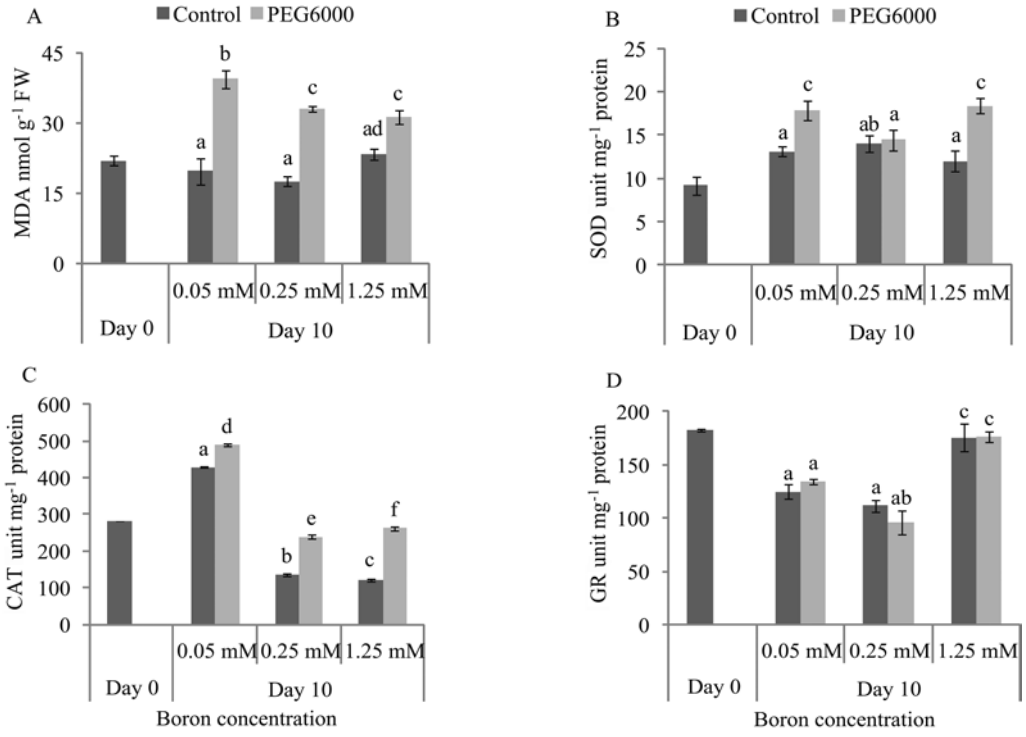
Lipid peroxidation level. The MDA content in leaves of 'Kar 98' did not change significantly at 0.25 mM B treatment, whereas a 19% enhancement occurred with 1.25 mM B dosage (Fig. 4 A). PEG 6000 treatment doubled MDA content in control plants with 0.05 mM B. Nevertheless, 0.25 and 1.25 mM B dosages alleviated the increase in MDA content caused by PEG 6000-induced drought stress by decreasing MDA by 16% and 20%, respectively. Although increasing dosages of B treatments mitigated the harmful effect of drought stress on lipid peroxidation level, MDA contents in PEG 6000-treated groups were still higher than in respective groups without PEG 6000 treatment regardless of B

dosages applied. The 100% increase in lipid peroxidation level induced by PEG 6000-induced drought stress alone in control plants (0.05 mM B) was significantly reduced by 0.25 and 1.25 mM B treatments (Fig. 4 A). This implies that H_2O_2 level in leaves of watermelon plants forms a reliable parameter to assess the degree of stress caused by the combination of drought stress and boron toxicity. Also, high dosages of B treatments lessened peroxidation of lipid membranes implied the positive effect on drought resistance mechanism of watermelon plants especially under water scarce conditions. This supports the importance of antioxidant response in watermelon plants as well under similar conditions.

Antioxidant enzyme activities. Superoxide dismutase (SOD) activity. The SOD activity in leaves was 7% enhanced after 0.25 mM B treatment, but not significantly changed by 1.25 mM B relative to the control group (containing 0.05 mM B). PEG 6000-induced drought stress caused a 37% enhancement in SOD activity in the control plants. Under drought stress the 0.25 mM B treatment resulted in a 19% decrease in SOD activity, whereas 1.25 mM B dosage did not affect SOD activity significantly in comparison to 0.05 mM B treatment (control group). Drought stress seemed to enhance SOD activity by 0.05 mM (control group, 37% increase) and 1.25 mM boron treatments (a 53% increase) compared to respective groups without PEG 6000 treatment (Fig. 4 B).

Catalase (CAT) activity. The CAT activities were decreased by 68% and 72% after 0.25 and 1.25 mM B treatments, respectively (Fig. 4 C). In contrast, drought stress caused a 14% increase in CAT activity in the control plants with 0.05 mM B dosage. Increasing B dosages up to 0.25 and 1.25 mM, respectively, resulted in 51% and 46% decline in CAT activity under PEG 6000-induced drought stress. The CAT activities in leaves of 'Kar 98' under PEG 6000-induced drought stress were 76% and 118% higher in unstressed groups treated with 0.25 and 1.25 mM boron, respectively.

Glutathione reductase (GR) activity. Although 0.25 mM B treatment did not change GR activity, 1.25 mM B caused a 40% increase in GR activity in leaves of 'Kar 98' (Fig. 4 D). Similarly, 0.200 mM B did not cause any change in GR activity in wheat leaves (Masood et al., 2012). However, Keleş et al. (2011) showed that 20 and 40 μg g^{-1} soil B treatments increased GR activity in shoots and leaf tissues of sunflower. Except for a slight (14%) decrease in 0.25 mM B treatment, PEG 6000-induced drought stress did not cause remarkable change in GR activity in 0.25 and 1.25 mM B treatment groups. The 0.25 and 1.25 mM B dosages resulted in 29% decrease and 31% increase in GR activity under drought stress conditions. 5 mM B increased GR activity of a barley cultivar sensitive to excess B (Karabal et al., 2003). Tombuloglu et al. (2012) exposed tomato plants to B dosages from 0.08 to 5.12 mM for 24 h and measured the expression levels of *GR1* gene among some stress-related genes. They showed that accumulation of *GR1* transcripts started to increase at 80 μM and reached a peak at 160 μM and at B dosages higher than 320 μM, *GR1* dramatically decreased. Their findings suggested that activation of *GR1* initially acted as a protection mechanism against B stress to regulate cellular homeostasis as an early response but due to possible cellular damage at higher B dosages, the GR pathway was not involved in resistance mechanism



Notes. Values are the mean ± SE of two independent experiments (n ≥ 6). Means followed by the same letters are not significantly different at $P < 0.05$.

Figure 4. Effect of increasing boron dosages on malondialdehyde (MDA) content (A), superoxide dismutase (SOD) activity (B), catalase (CAT) activity (C) and glutathione reductase (GR) activity (D) of watermelon genotype 'Kar 98' before (day 0) and after exposure to PEG 6000-induced drought stress for 10 days (day 10)

to ameliorate B stress (Tombuloglu et al., 2012). Thus, B treatment seems to induce GR activity at transcriptional level as well, which suggests that GR activity induction in leaves of 'Kar 98' occurred at 1.25 mM B might serve as a resistance mechanism in watermelon plants against toxic B stress in the presence or absence of drought stress induced by PEG 6000.

In consonance with the results of Cakmak and Römheld (1997) and Karabal et al. (2003), who reported no change in SOD activities in leaves of sunflower and barley, respectively, increasing B dosages did not cause remarkable change in SOD activity of watermelon genotype 'Kar 98', in the present study. Yildiztugay et al. (2014) showed enhancement in SOD activity, in roots of maize plants under PEG-induced severe water stress. Similarly, SOD activity of watermelon plants was enhanced by PEG 6000-induced water stress alone in comparison to control (applied with 0.05 mM B and no PEG 6000) plants. The parallel trends and positive correlation between H_2O_2 accumulation level and SOD activity occurred with increasing levels of B under PEG 6000-induced drought stress suggest that H_2O_2 production was related to conversion of O_2^- to H_2O_2, by SOD activity in leaves of watermelon plants. CATs are responsible for bulk removal of H_2O_2 as they have very high turnover rate but quite low affinity towards H_2O_2. The effects of B toxicity on CAT activity differ among plant species and different changes in its activities are reported in the literature. For instance, although B toxicity decreased CAT activity in barley (Inal et al., 2009) and in tomato and sunflower (Keleş et al., 2011), it enhanced the CAT activity in sunflower (Karabal et al., 2003), tomato (Cervilla et al., 2007) and chickpea (Ardic et al., 2009 a; b). The differences in the responses of the same species may be attributed to the different genotypes used in consecutive studies. Increasing dosages of B caused remarkable decrease in CAT activity of watermelon plants and PEG 6000-induced drought stress promoted it although toxic B dosages prevented increase in CAT activity. In previous studies, 20% and 40% PEG 6000 treatments also enhanced CAT activity in maize roots (Yildiztugay et al., 2014).

Conclusions

1. Increasing boron (B) dosages produced more dramatic growth reduction and oxidative stress than the combination of B toxicity and PEG 6000-induced drought stress. Less intake of B into watermelon plants due to restricted water uptake through PEG 6000-induced drought stress seemed to alleviate the deleterious effects of excess B accumulation on plant growth.

2. Boron toxicity caused peroxidation of lipid membranes independent of changes in reactive oxygen species (ROS) activity.

3. Addition of higher B in the presence of drought stress reduced malondialdehyde (MDA) content and induced glutathione reductase (GR) activity demonstrating that increased GR activity is an important physiological response in watermelon plants against the combination of B toxicity and drought stress.

The results of this study have significance in modelling and management of drought stress in watermelon plants under high B conditions. The effect of B toxicity in combination with drought stress on non-enzymatic antioxidants, photosynthetic efficiency and yield are worthy of future research.

Acknowledgements

We gratefully acknowledge funding support from the Scientific Research Project Commission of Selçuk University. We also thank to Prof. Dr. Nebahat Sari and Dr. İlknur Solmaz from Çukurova University, Department of Horticulture for supplying watermelon seeds.

Received 20 11 2014
Accepted 16 04 2015

References

Ardic M., Sekmen A. H., Turkan I., Tokur S., Ozdemir F. 2009 (a). The effects of boron toxicity on root antioxidant systems of two chickpea (*Cicer arietinum* L.) cultivars. Plant and Soil, 314: 99–108 http://dx.doi.org/10.1007/s11104-008-9709-y

Ardic M., Sekmen A. H., Tokur S., Ozdemir F., Turkan I. 2009 (b). Antioxidant responses of chickpea plants subjected to boron toxicity. Plant Biology, 11: 328–338 http://dx.doi.org/10.1111/j.1438-8677.2008.00132.x

Beauchamp C., Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44: 276–287 http://dx.doi.org/10.1016/0003-2697(71)90370-8

Ben-Gal A., Shani U. 2002. Yield, transpiration and growth of tomatoes under combined excess boron and salinity stress. Plant and Soil, 247: 211–221 http://dx.doi.org/10.1023/A:1021556808595

Bradford M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the protein-dye binding. Analytical Biochemistry, 72: 248–254 http://dx.doi.org/10.1016/0003-2697(76)90527-3

Cakmak I., Römheld V. 1997. Boron deficiency-induced impairments of cellular functions in plants. Plant and Soil, 193: 71–83 http://dx.doi.org/10.1023/A:1004259808322

Cervilla L. M., Blasco B., Rios J. J., Romero L., Ruiz J. M. 2007. Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to boron toxicity. Annals of Botany, 100: 747–756 http://dx.doi.org/10.1093/aob/mcm156

Cervilla L. M., Rosales M. A., Rubio-Wilhelmi M. M., Sánchez-Rodríguez E., Blasco B., Ríos J. J., Romero L., Ruiz J. M. 2009. Involvement of lignification and membrane permeability in the tomato root response to boron toxicity. Plant Science, 176: 545–552 http://dx.doi.org/10.1016/j.plantsci.2009.01.008

Cervilla L. M., Blasco B., Rios J. J., Rosales M. A., Sanchez-Rodriguez E., Rubio-Wilhelmi M. M., Romero L., Ruiz J. M. 2012. Parameters symptomatic for boron toxicity in leaves of tomato plants. Journal of Botany, 17 p. http://dx.doi.org/10.1155/2012/726206

Chung S. K., Osawa T., Kawakishi S. 1997. Hydroxyl radical-scavenging effects of spices and scavengers from brown mustard (*Brassica nigra*). Bioscience, Biotechnology, and Biochemistry, 61: 118–123 http://dx.doi.org/10.1271/bbb.61.118

Coskun Y., Olgunsoy P., Karatas N., Bulut F., Yarar F. 2014. Mannitol application alleviates boron toxicity in wheat seedlings. Communications in Soil Science and Plant Analysis, 45: 944-952 http://dx.doi.org/10.1080/00103624.2013.867054

FAO. 2012. The state of food and agriculture. Investing in agriculture for a better future. <http://www.fao.org/docrep/017/i3028e/i3028e.pdf> [accessed 14 03 2014]

Foyer C. H., Halliwell B. 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta, 133: 21–25 http://dx.doi.org/10.1007/BF00386001

Hoagland D. R., Arnon D. I. 1950. The water-culture method for growing plants without soil. Circular 347. University of California Agricultural Experimental Station, Berkeley, USA, p. 1–39

Inal A., Pilbeam D. J., Gunes A. 2009. Silicon increases tolerance to boron toxicity and reduces oxidative damage in barley. Journal of Plant Nutrition, 32: 112–128 http://dx.doi.org/10.1080/01904160802533767

Karabal E., Yucel M., Oktem H. A. 2003. Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. Plant Science, 164: 925–933 http://dx.doi.org/10.1016/S0168-9452(03)00067-0

Keleş Y., Ergün N., Öncel I. 2011. Antioxidant enzyme activity affected by high boron concentration in sunflower and tomato seedlings. Communications in Soil Science and Plant Analysis, 42 (2): 173–183 http://dx.doi.org/10.1080/00103624.2011.535068

Landi M., Pardossi A., Remorini D., Guidi L. 2013. Antioxidant and photosynthetic response of a purple-leaved and a green-leaved cultivar of sweet basil (*Ocimum basilicum*) to boron excess. Environmental and Experimental Botany, 85: 64–75 http://dx.doi.org/10.1016/j.envexpbot.2012.08.008

Madhava Rao K. V., Sresty T. V. S. 2000. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Science, 157 (1): 113–128 http://dx.doi.org/10.1016/S0168-9452(00)00273-9

Masood S., Saleh L., Witzel K., Plieth C., Mühling K. H. 2012. Determination of oxidative stress in wheat leaves as influenced by boron toxicity and NaCl stress. Plant Physiology and Biochemistry, 56: 56–61 http://dx.doi.org/10.1016/j.plaphy.2012.04.011

Pandey N., Archana. 2013. Antioxidant responses and water status in *Brassica* seedlings subjected to boron stress. Acta Physiologiae Plantarum, 35 (3): 697–706 http://dx.doi.org/10.1007/s11738-012-1110-z

Rahal A., Kumar A., Singh V., Yadav B., Tiwari R., Chakraborty S., Dhama K. 2014. Oxidative stress, prooxidants, and antioxidants: the interplay. BioMed Research International, 19 p. http://dx.doi.org/10.1155/2014/761264

Rao M. V., Paliyath G., Ormrod D. P., Murr D. P., Watkins C. B. 1997. Influence of salicylic acid on H_2O_2 production, oxidative stress, and H_2O_2-metabolizing enzymes: salicylic acid-mediated oxidative damage requires H_2O_2. Plant Physiology, 115 (1): 137–149 http://dx.doi.org/10.1104/pp.115.1.137

Rouphael Y., Cardarelli M., Colla G. 2008. Yield, mineral composition, water relations, and water use efficiency of grafted mini-watermelon plants under deficit irrigation. HortScience, 43: 730–736

Siddiqui M. H., Al-Whaibi M. H., Sakran A. M., Ali H. M., Basalah M. O., Faisal M., Alatar A., Al-Amri A. A. 2013. Calcium-induced amelioration of boron toxicity in radish. Journal of Plant Growth Regulation, 32: 61–71 http://dx.doi.org/10.1007/s00344-012-9276-6

Tombuloglu H., Semizoglu N., Sakcali S., Kekec G. 2012. Boron induced expression of some stress-related genes in tomato. Chemosphere, 86: 433–438 http://dx.doi.org/10.1016/j.chemosphere.2011.09.035

Türkan I., Bor M., Özdemir F., Koca H. 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Science, 168: 223–231 http://dx.doi.org/10.1016/j.plantsci.2004.07.032

Velikova V., Yordanov I., Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants protective role of exogenous polyamines. Plant Science, 151: 59–66 http://dx.doi.org/10.1016/S0168-9452(99)00197-1

Yildiztugay E., Ozfidan-Konakci C., Kucukoduk M. 2014. Exogenous nitric oxide (as sodium nitroprusside) ameliorates polyethylene glycol-induced osmotic stress in hydroponically grown maize roots. Journal of Plant Growth Regulation, 33: 683–696 http://dx.doi.org/10.1007/s00344-014-9417-1

Yokota A., Kawasaki S., Iwano M., Nakamura C., Miyake C., Akashi K. 2002. Citrulline and DRIP-1 protein (ArgE homologue) in drought tolerance of wild watermelon. Annals of Botany, 89: 825-832 http://dx.doi.org/10.1093/aob/mcf074

ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 102, No. 2 (2015), p. 209–216
DOI 10.13080/z-a.2015.102.027

Arbūzo (*Citrullus lanatus*) antioksidacinis stresas, priklausomai nuo boro koncentracijos ir sausros

M. Hamurcu[1], T. Demiral[2], E. E. Hakki[1], Ö. Turkmen[2], S. Gezgin[1], R. W. Bell[3]

[1]Selçuk universitetas, Turkija
[2]Harran universitetas, Turkija
[3]Vakarų Australijos Murdocho universiteto Aplinkos mokslų mokykla

Santrauka

Tyrimo tikslas – ištirti įvairių boro (B) koncentracijų įtaką arbūzų atsparumui sausrai. Sausrai atsparus tikrojo arbūzo (*Citrullus lanatus* (Thunb.) Matsum. et Nakai) genotipas ‘Kar 98’ buvo auginamas kontroliuojamomis šiltnamio sąlygomis hidroponiškai ir veikiamas sausros stresu, taikant PEG 6000 (polietilenglikolį) ir naudojant tris boro dozes: 0,05, 0,25 bei 1,25 mM. Buvo nustatyti šie augimo rodikliai: žalios masės svoris, sausos masės svoris ir daigų bei šaknų ilgis, santykinis drėgmės kiekis lape, boro susikaupimas, lipidų peroksidacijos lygis ir superoxidazės dismutazės, katalazės bei glutationo reduktazės fermentų aktyvumas; taip pat buvo įvertintas H_2O_2 susikaupimas ir •OH-radikalų surišimas. Didesnės boro dozės sukėlė r.yškesnį arbūzų augimo sumažėjimą, palyginus su jų ir PEG 6000 derinio sukeltu stresu. Dėl sausros sukelto streso mažiau boro susikaupė lapuose ir šaknyse. Nepriklausomai nuo reaktyviųjų deguonies junginių poveikio, boro 1.25 mM koncentracija sukėlė lipidų peroksidaciją, o boro dozėms didėjant sausros sukelta lipidų peroksidacija mažėjo. Glutationo reduktazės aktyvumo paveikus 1.25 mM boro ir PEG 6000 sukeltas stresas pasirodė svarbus genotipo ‘Kar 98’ augalų fiziologinis atsakas į daugybinį stresą.

Reikšminiai žodžiai: antioksidacinis aktyvumas, boro kaupimasis, lipidų peroksidacija, PEG 6000, vandenilio peroksidas.