

Full Length Research Paper

Antioxidant responses of lentil and barley plants to boron toxicity under different nitrogen sources

Muammer Tepe^{2*} and Tülin Aydemir¹

¹Prof. Tülin Aydemir, Celal Bayar University Science and Arts Faculty Chemistry Department, Turkey.

²Muammer Tepe; High Biochemist, Celal Bayar University Science and Arts Faculty, Turkey.

Accepted 2 June, 2011

In this study, the effects of different nitrogen sources on lentil (*Lens clunaris*) and barley (*Hordeum vulgare*) plants, exposed to 5 and 10 mM boron stress previously, were studied. After ten-day germination, the lentil (native) and barley (Tokak157/37) were incubated 16 h light and 8 h dark per day for 7-day growth cycle under the conditions of boron stress via different nitrogen sources (10 mM nitrogen in NH₄ Cl, KNO₃ and urea). As a result of the changes in the nitrogen sources of the plants, there were determined decreases in the relative growth rate (%) and total chlorophyll content related to boron stress, ($p < 0.05$) and ($p < 0.01$), respectively. The changes in the lentil were obtained much more than those in the barley. In addition, the changes in the groups in which NH₄⁺ was used as nitrogen source were obtained at lowest levels. The concentrations of MDA, H₂O₂ and proline showed increases under boron stress ($p < 0.05$). The effect of boron toxicity on the activities of SOD, GPX and LOX was similar in the two species but the levels of CAT and APX activities were different in both species under 5 and 10 mM boron stress ($p < 0.01$). SOD, GPX and LOX activities increased in the roots and shoots of boron treated plants as compared with the controls ($p < 0.01$). Although, lentil CAT and APX, activities decreased; in barley, CAT and APX, activities increased under boron toxicity. In conclusion, the fertilizers which contain NH₄⁺ should be used in the boron stressed farmlands.

Key words: Boron toxicity, antioxidant enzymes, nitrate, ammonium, urea, lentil, barley.

INTRODUCTION

Boron (B) which is widely distributed in the lithosphere and hydrosphere is an essential plant micronutrient that can be phytotoxic to plants when it is present in soils and waters at high concentrations (Nable et al., 1997). Boron is an important constituent of the cell walls and has been reported to be involved in enzyme activation, membrane maintenance, nucleic acid metabolism and sugar translocation (Dordas and Brown, 2000). Boron toxicity symptoms were identified, usually in grains, in many countries of West Asia-North Africa; for example,

Anatolian Plateau of Turkey, areas around Aleppo in Syria (Yau et al., 1996) and on the northwest coast of Egypt. In Turkey, Boron toxicity symptoms were detected in farmers' fields in the Ankara, Konya and Eskisehir areas and at the Hamidiye Research Station near Eskisehir (Rathjen, personal communication, 1992; Yau et al., 1996). Plants exposed to boron toxicity include reduced vigour, delayed development, leaf symptoms (yellowing of leaf tips of older leaves followed by non-specific necrosis continuing down the leaves and reduced total dry matter) and finally, decreased grain yields (Nable et al., 1997). Boron is involved in a number of metabolic pathways and functions such as cell wall synthesis and structure, lignification, carbohydrate metabolism, phenol metabolism and plasma membrane integrity (Dordas and Brown, 2000).

When plants are subjected to boron toxicity, such biochemical changes occur as production of reactive oxygen species (ROS) like superoxide, hydrogen peroxide and hydroxyl radicals (Dat et al., 2000). In order to avoid the harmful effects of these ROS, plants evolved

*Corresponding author. E-mail: debtepoglu@hotmail.com.

Abbreviations: SOD, Superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GPX, guaiacol peroxidase; PEG, polyethylene glycol; AOS, active oxygen species; NBT, nitroblue tetrazolium; EDTA, ethylenediamine-N,N,N',N'-tetraacetic acid; MDA, malondialdehyde; DAB, diamino-benzidine tetrahydrochloride; DW, dry weight; FW, fresh weight.

an effective scavenging system composed of non-enzymatic antioxidants (carotenoids, ascorbate, tocopherol) and enzymatic antioxidants, such as catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11), lipoxygenase (LOX; EC 1.13.11.12) and guaiacol peroxidase (GPX; EC 1.11.1.7). The results of most studies suggest that the resistance to salt stress is usually correlated with a more efficient antioxidative system (Gossett et al., 1994). The enzymatic action of APX reduces H_2O_2 using the ascorbate as an electron donor. CAT is also implicated in removal of H_2O_2 . Salinity induced production of ROS disturbs the cellular redox system in favor of oxidized forms, thereby, creating an oxidative stress that can damage DNA, inactivate enzymes and cause lipid peroxidation (Smirnoff, 1993).

Nitrogen (N) is an essential element in biological materials and changes in its availability and metabolism are of particular importance for maintaining high productivity of crops (Ullrich, 2002). Nitrogen is available to the plant in two forms-ammonium (NH_4^+) and nitrate (NO_3^-). In most soils, ammonium is quickly converted to the nitrate form, a process called nitrification. This nitrate form is not tightly held on soil particles and is soluble in water. Consequently, nitrogen management is important both from a production and environmental standpoint. Urea is ubiquitous in natural environments and serves as readily available nitrogen (N)-source for the growth of many organisms, including plants. It is synthesized during the degradation of amino acids and purines and is accumulated in source tissues such as senescing leaves and germinating seeds, which remobilize N to sustain growth in metabolic sinks. Urea is also an N source provided either from fertilizer or from grazing livestock and although, most of it is hydrolysed in the soil, the direct use of leaf-applied urea is a promising development due to the high tolerance of plants to this non-ionic form of N; it has the lowest osmolality among all N sources (Weast, 1978) and because of its zero charge, it is not accompanied by ions that tend to accumulate in solution. The assimilation of NO_3^- by plants requires the uptake of NO_3^- , reduction to NO_2^- , the conversion of NO_2^- to ammonium (NH_4^+) and the incorporation of NH_4^+ into organic compounds (Migge and Becker, 1996). This process is complex and is regulated by a number of physiological and environmental factors such as NO_3^- , availability, cold, salt and light. Several studies have also implicated B in N metabolism. Boron has been shown to be essential in fixing N in *Cyanobacteria heterocysts* (Bonilla et al., 1990). Many soils contain large amounts of nitrogen, but most of the nitrogen is tied up in the organic fraction and only slowly released. Boron is also involved in nitrogen fixation. Loomis and Durst (1992) reported that boron is an essential micronutrient required for growth and development of vascular plants, diatoms and species of marine algal flagellates, while bacteria, fungi, green algae and animals apparently do not require boron.

The antioxidative defence system enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase

(CAT), guaiacol peroxidase (GPX) and lipoxygenase (LOX) were measured in crude extracts of the roots and shoots of lentil and barley plants. On the other hand, this study determined the effects of N forms such as NO_3^- , NH_4^+ or urea on the growth, photosynthetic pigment content and MDA, H_2O_2 and proline content of lentil and barley plants under different boron concentration.

MATERIALS AND METHODS

Plant material and growth conditions

In this study, Turkish cultivars of barley (*Hordeum vulgare* L.), tokak 157/37 and Lentil (*Lens culinaris*) native, were used. The barley seeds were obtained from the Turkish Ministry of Agriculture and Rural Affairs, Central Research Institute for Field Crops, Ankara and the lentil seeds were obtained from Southeastern Anatolia Agricultural Research Institute. Seeds were first surface-sterilized with 30% sodium hypochloride solution for 10 min. Then, they were washed and imbibed in distilled water for 1 day. After imbibition, approximately 15 to 20 seeds were planted onto plastic trays covered with filter paper and cotton containing half-strength Hoagland's solution. They were grown for 10 days in a growth chamber at $23\pm 2^\circ C$ with 16-h light: 8-h dark photo-cycle at a light intensity of $40\text{ mmol m}^{-2}\text{ s}^{-1}$.

At the 10th day of growth, boron stress treatment was initiated by applying half-strength Hoagland's solution containing 5 or 10 mM boric acid (H_3BO_3) and different nitrogen sources ($NH_4\text{Cl}$, KNO_3 , urea are containing 10 mM nitrogen) to seedlings. Control plants (no boric acid treatment) and boron stressed plants with different nitrogen sources were grown in the growth chamber with the same physical parameters for another 7 days. The shoot and root tissues of 17 days old seedlings were then freshly used in the experiments.

Growth measurements

After 0 and 7 days of boron treatment, 1 g plants for each group were taken at random and divided into separate shoot and root fractions. The fresh weights of shoots and roots were weighed. After measuring the fresh weights of seedlings, the same tissues were let to dry in an oven at $70^\circ C$ for 24 h and then the dry weights of the samples were recorded. The relative growth rate was calculated as the ratio of fresh weight difference between fresh weight at the end of 7 days of incubation and fresh weight at the beginning of the experiment over fresh weight at the beginning of the experiment $[\ln(W_{t_7} - W_{t_0}) : t_7 - t_0] \times 100$. For each treatment, 15 to 20 seedlings were used and the experiment was repeated five times.

Determination of proline and H_2O_2 content

The amount of proline was determined according to a modified method of Bates et al. (1973) Approximately, 0.5 g of shoot and root tissues from control and treated plants were cut into small pieces and homogenized by the addition of 1 ml of 5-sulphosalicylic acid solution in ice bath. The homogenates were centrifuged at 13 000 rpm for 10 min at $4^\circ C$.

For each sample, an Eppendorf tube containing 0.2 ml acid ninhydrin (0.31 g ninhydrin, 7.5 ml acetic acid and 5 ml 6 M phosphoric acid), 0.2 ml 96% acetic acid and 0.1 ml 3% 5-sulphosalicylic acid were prepared. Supernatant (0.1 ml) from each homogenate was added to the tubes. Tubes were incubated at

96°C for 1 h in a hot block and after incubation, 1 ml of toluene were added to each tube. Then, tubes were mixed and centrifuged at 13 000 rpm for 10 min at 4°C. The toluene phase was carefully pipetted out into a glass test tube and the absorbance was measured at 520 nm in a spectrophotometer. The concentration of proline was calculated from a proline standard curve. The concentration of proline was expressed as $\mu\text{mol/g FW}$.

The hydrogen peroxide content was determined according to Jana and Choudhuri (1981). Aliquots of fresh shoots and roots were homogenized in 50 mM potassium phosphate, pH 6.5 and centrifuged at $10\,000 \times g$ for 25 min. The solution was mixed with 1% titanium chloride (in concentrated HCl) and then centrifuged at $10\,000 \times g$ for 15 min. The absorbance of the supernatant was measured at 410 nm and the H_2O_2 content was calculated using $0.28 \mu\text{M}^{-1}\text{cm}^{-1}$ as extinction coefficient.

Determination of MDA content

MDA content was determined spectrophotometrically as described by Heat and Packer (1968). Samples were homogenized in 1% trichloroacetic acid and then centrifuged at 10,000 rpm for 15 min. Supernatant was heated with 0.05 thiobarbituric acid for 30 min at 95°C. The heated supernatant was recentrifuged at 5000 rpm for 5 min and the absorbance was measured at 532 and 600 nm on UV-VIS Spectrometer (UV-Vis 530, Jasco, Japanese). The non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance. The absorbance coefficient of MDA $155 \text{ mM}^{-1} \text{ cm}^{-1}$ was used in the calculation accordingly.

Chlorophylls contents

Chlorophyll contents were estimated by extracting 0.05 g of the leaf material in 10 ml dimethylsulfoxide (DMSO). The samples were heated at 65°C for 4 h and then the absorbance of extract was recorded at 665, 645 and 470 nm. Pigment concentrations were calculated according to the procedure described by Lichtenthaler and Wellburn (1983). Results have been expressed as mg chlorophyll or carotenoids mg/g fresh weight.

Enzyme extractions and assays

Fresh shoot and root samples weighting about 1 g were homogenized using chilled mortar and pestle in 5 ml of cold 20 mM potassium phosphate buffer (pH 7.0) containing 1.0 % insoluble polyvinyl pyrrolidone (PVP) in ice bath. The homogenates were centrifuged at $12\,000 g$ for 30 min. The supernatant was stored at 4°C and used for enzyme assays.

POD (Guaiacol peroxidase-EC. 1.11.1.7) activity was measured by the increase in absorbance at 470 nm due to guaiacol oxidation. The reaction mixture contained 25 mM guaiacol, 10 mM H_2O_2 and 0.1 ml supernatant. The reaction was started by adding hydrogen peroxide. Enzyme activity was determined using and extinction coefficient of $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ (Nakano and Asada, 1981).

CAT (Catalase-EC 1.11.1.6) activity was measured spectrophotometrically at 240 nm according to the method of Aebi (1984). One unit of the catalase activity is defined as the activity required to destroy 1 μmol hydrogen peroxide in 50 mM phosphate buffer (pH 7.0) at 25°C in 30 s.

SOD (superoxide dismutase-EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NTB) according to the method of Beyer and Fridovich (1987). Leaf samples were homogenized in four volumes (w/v) of an ice-cold buffer containing 0.1 M Tris-HCl (pH 7.8), 0.1 mM EDTA and 0.05% Triton X-100. The homogenates were filtered through four layers of cheesecloth and centrifuged at 4°C for 30 min

at $15\,000 \times g$. The crude extracts were dialyzed for 24 h against half strength extraction buffer without Triton X-100, centrifuged for 20 min at $15\,000 \times g$ and the supernatants were used for SOD assay. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.053 mM NTB, 10 mM methionine, 0.0053 mM riboflavin and an appropriate aliquot of enzyme extract. The reaction was started by switching on the light and allowed to run for 7 min. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NTB as monitored at 560 nm. The activity of SOD was expressed as Unit/g fresh weight.

APX (Ascorbate peroxidase-EC. 1.11.1.11) activity was measured immediately in fresh extracts and was assayed as describes by Nakano and Asada (1981) using a reaction mixture containing 25 mM potassium phosphate buffer (pH 7.0), 0.1 mM H_2O_2 , 0.5 mM ascorbate and 0.1 mM EDTA. The hydrogen peroxide-dependent oxidation was followed by a decrease in the absorbance at 290 nm. The activity of APX was calculated using the extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$.

LOX (Lipoxygenase-EC.1.13.11.12) activity was measured according to Axelrod (1981). The reaction was initiated by addition of 0.2 ml enzyme extract in 4 ml of reaction mixture containing 50 mM sodium phosphate buffer (pH 6.5) and 0.4 mM linoleic acid. The absorbance was recorded at 234 nm. The activity was calculated using the extinction coefficient ($25 \text{ mM}^{-1} \text{ cm}^{-1}$) for H_2O_2 .

Statistical analysis

All data presented are the mean values. The measurement was done with three replicates on plant growth data and heavy metals accumulation, five replicates on all anti-oxidative enzyme activities, MDA, Proline and H_2O_2 . Statistical assays were carried out by one-way ANOVA using Student's *t*-test to test the different significance.

RESULTS

The effect of different nitrogen sources on growth parameters, certain antioxidant enzymes and total chlorophyll contents of lentil (*L. clunaris*) and barley (*H. vulgare*) under boron toxicity were studied. The activities of antioxidant enzymes including superoxide dismutase, SOD; ascorbate peroxidase, APX; catalase, CAT; lipoxygenase LOX; guaiacol peroxidase, GPX, were investigated in shoots and in roots of lentil (*L. clunaris*) and barley (*H. vulgare*) under the conditions of different nitrogen sources (KNO_3 , NH_4Cl and urea) and boron toxicity, in the absence (no boric acid treatments) and in the presence of 5 and 10 mM B in the nutrient solution.

Effect of boron toxicity and different nitrogen sources on plants growth

Using NH_4^+ as the nitrogen source, lentil and barley when compared with each other in the control groups showed the highest relative % growth rate ($p < 0.05$). Lentil and barley plants accumulated less biomass over the same growth period when receiving NO_3^- compared with NH_4^+ and urea (Figures 1 and 2). Boron toxicity affected the growth of roots more than the growth of shoots. The fresh and dry weights of shoots and roots were found to be the highest in NH_4^+ fed plants compared with the NO_3^- and

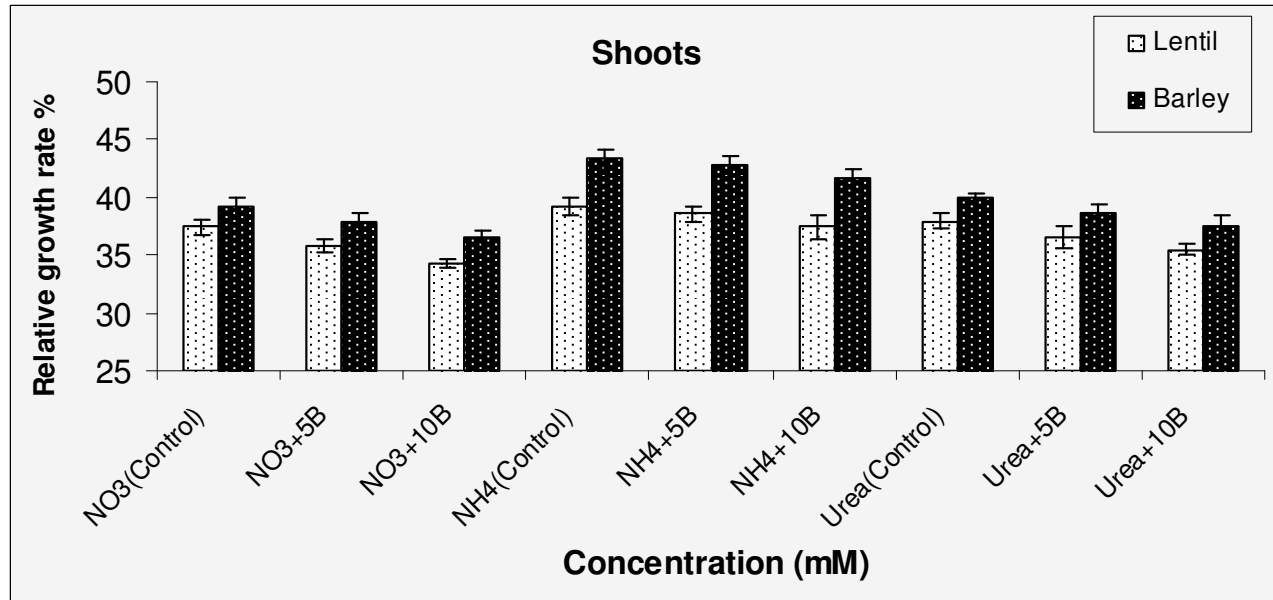


Figure 1. Relative growth rates % $[\ln(W_{t_7}-W_{t_0}):t_7-t_0]*100$ of entire plants, shoots of lentil and barley plants as affected by boron toxicity and different nitrogen sources. Bars represent the standard error.

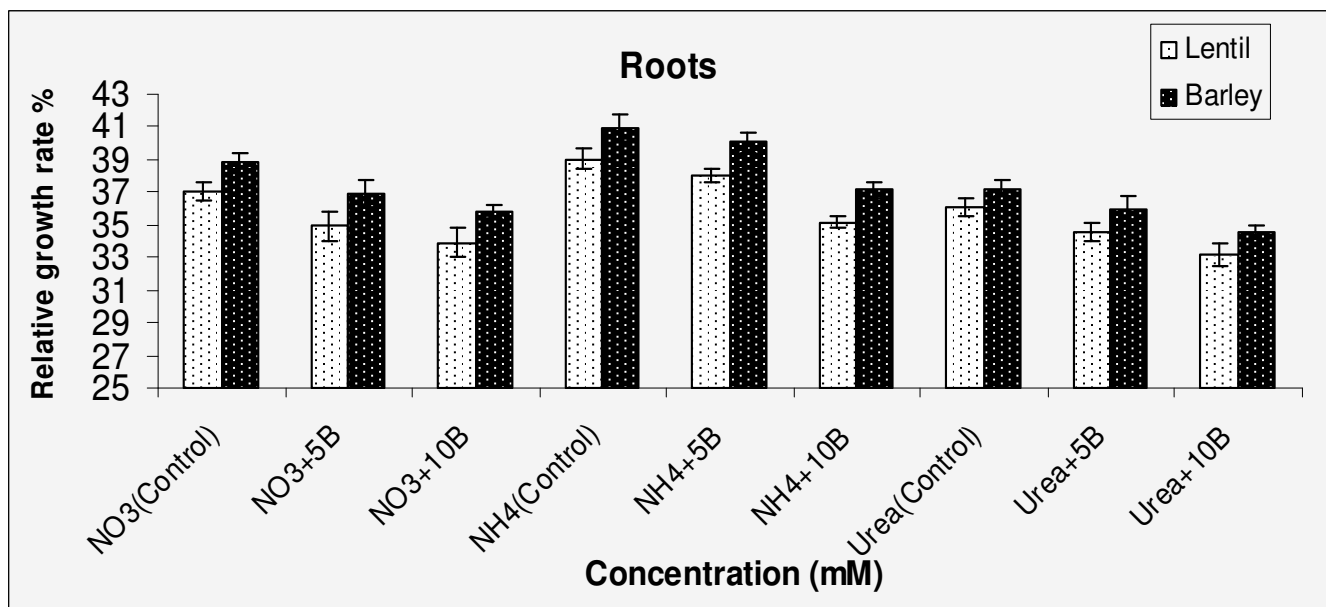


Figure 2. Relative growth rates % $[\ln(W_{t_7}-W_{t_0}):t_7-t_0]*100$ of entire plants, roots of lentil and barley plants as affected by boron toxicity and different nitrogen sources. Bars represent the standard error.

urea fed plants. Both tissues of barley 5 mM boron toxicity stress did not cause a significant difference, while 10 mM boron caused significant decreases in fresh and dry weights of shoot and root tissues of barley (Tokak 157/37) when compared with controls. On the contrary, boron toxicity (5 and 10 mM) caused significant decreases in fresh and dry weights of shoot and root tissues of Lentil (Native).

Effect of boron toxicity and different nitrogen sources on antioxidant enzyme activities

Figure 3 shows the activities of SOD, CAT, APX, GPX and LOX in lentil and barley under boron toxicity and different nitrogen sources. The effect of boron toxicity on the activities of SOD, GPX and LOX were similar in the two species. SOD, GPX and LOX activities increased in

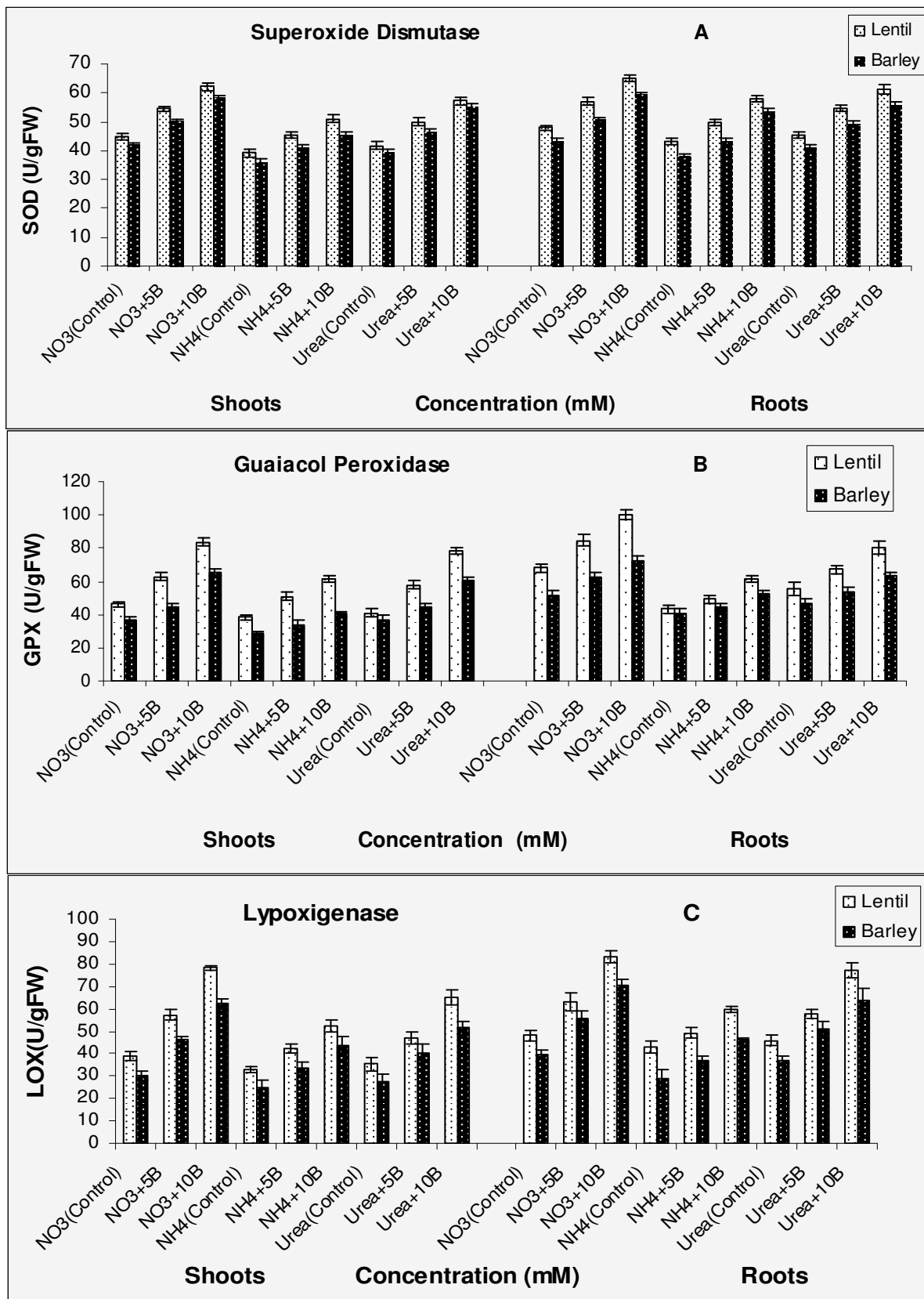


Figure 3. Developmental profiles of activities of SOD (A), GPX (B), LOX (C), APX (D), CAT (E) and in shoots and in roots during plant growth, as affected by boron toxicity and different nitrogen sources. Bars represent the standard error.

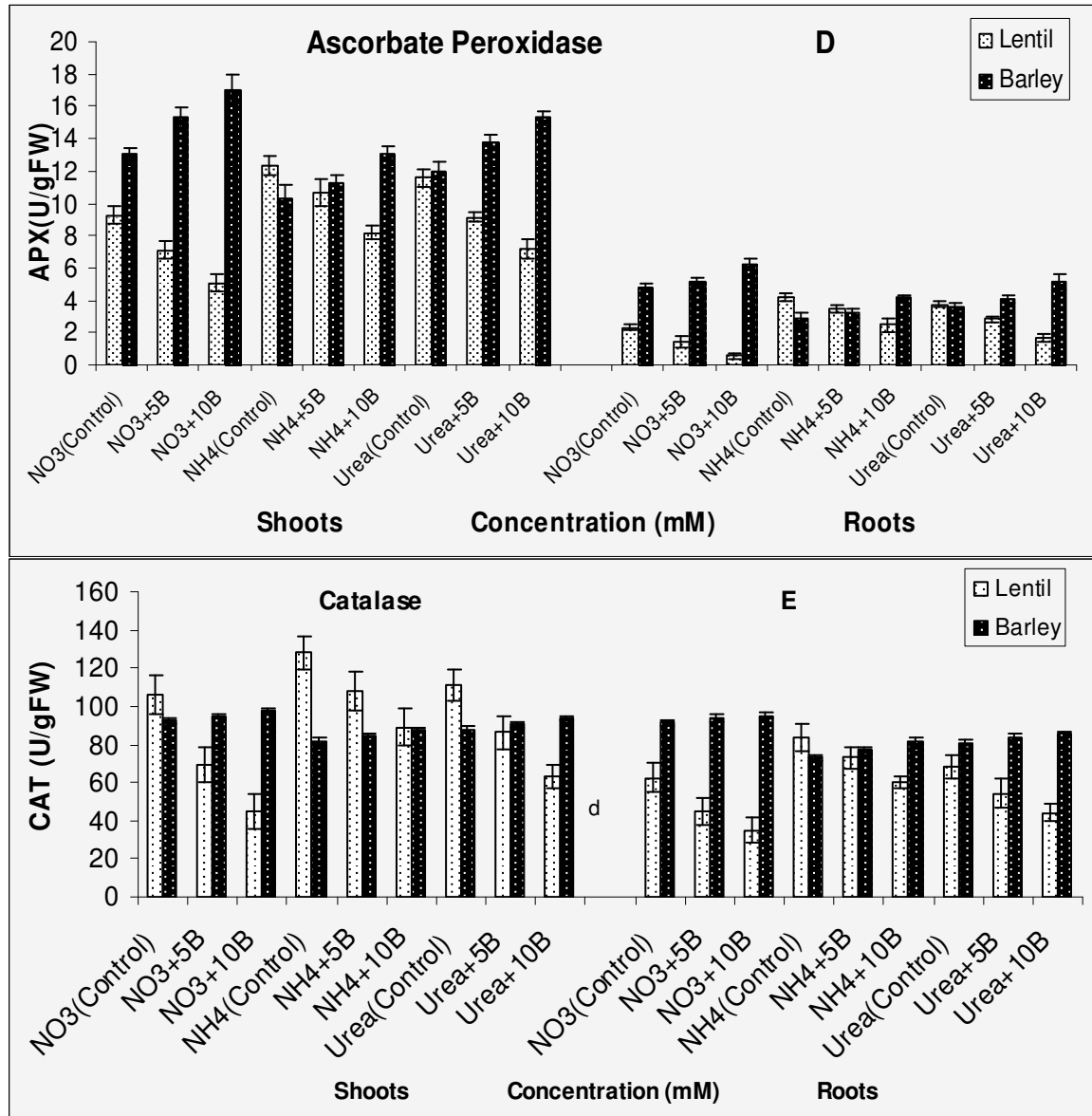


Figure 3. Contd.

the roots and shoots of boron treated plants when compared with the controls. The levels of CAT and APX activities were different in both species under 5 and 10 mM boron toxicity. Compared with controls, there were significantly higher APX and CAT activities in both tissues of barley, upon exposure to 5 and 10 mM boron toxicity treatments. In contrast, the activities of APX and CAT significantly decreased in lentil shoots and roots with increasing boron concentration when compared with their controls ($p < 0.01$). Nitrate-fed barley plants had higher SOD, GPX and LOX activities than ammonium and urea-fed plants. SOD, GPX and LOX activities were much higher in roots than in shoots of barley and lentil ($p < 0.01$). The highest CAT and APX activities were found in NH₄⁺ fed plants when compared with NO₃⁻ and urea-fed

plants ($p < 0.05$).

Effect of boron toxicity and different nitrogen sources on total chlorophyll content

Effects of 5 and 10 mM boron toxicity and different nitrogen sources on total chlorophyll contents of lentil and barley leaves are given in Figure 4. In the control groups when using NH₄⁺ as a nitrogen source, lentil and barley compared with each other showed the highest total chlorophyll contents ($p < 0.05$). Boron toxicity caused significant decrease in total chlorophyll contents of leaf tissues of both cultivars in a dose dependent manner. In leaf tissues of barley, 5 mM boron toxicity stress did not

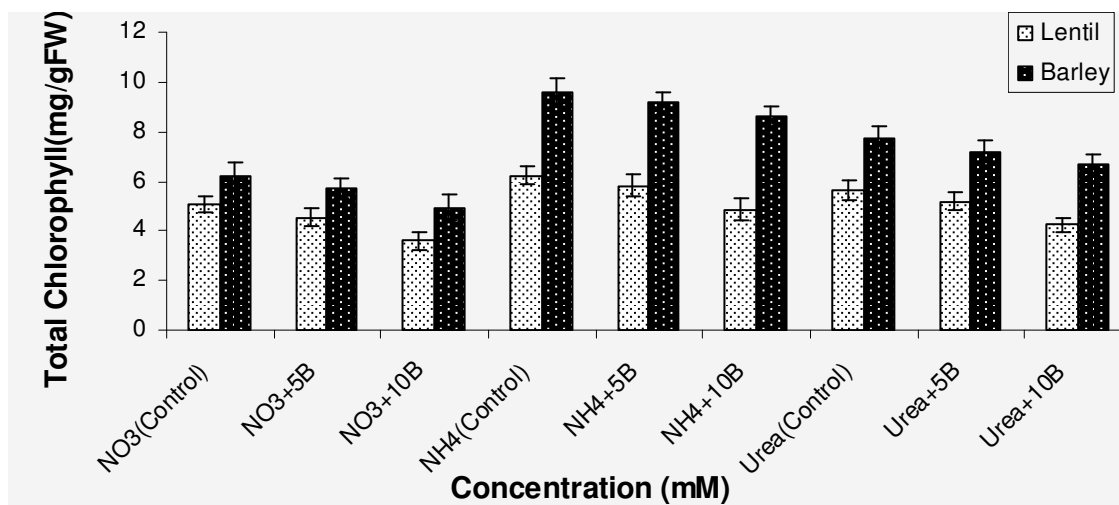


Figure 4. Effect of boron stress and different nitrogen sources on total chlorophyll contents of lentil and barley plants. Bars represent the standard error. The results are the mean \pm S.E. of three replicates.

cause a significant difference. In boron treated plant, the total chlorophyll contents were found to be maximal ($p < 0.05$) in NH_4^+ fed plants in all leaf tissues and there was the highest decrease in all tissues of NO_3^- fed plants.

Effect of boron toxicity and different nitrogen sources on MDA, H_2O_2 and proline contents

Effects of increasing level of boron toxicity and different nitrogen sources on MDA, H_2O_2 and proline content of lentil and barley plants are given in Table 1. In both of tissue of barley, 5 mM boron toxicity stress did not cause a significant difference. The level of MDA, H_2O_2 and proline content in lentil and barley increased in the presence of 10 mM boron toxicity with all of the nitrogen sources in comparison to the control groups (no boric acid treatments). The MDA, H_2O_2 and proline content was found to be maximal ($p < 0.05$) in NO_3^- fed plants in all shoot and root tissues and there was the lowest increase in all tissues of NH_4^+ fed plants. A significant increase ($p < 0.001$) MDA, H_2O_2 and proline accumulation was found in the roots of all treatments compared to shoots. On the other hand, when barley and lentil plants were compared, the highest increase in MDA, H_2O_2 and proline contents were obtained in 5 and 10 mM boron-treated lentil groups ($p < 0.01$)

DISCUSSION

In plants, boron is known to play important roles in structure of cell-walls, membranes and membrane associated reaction. The growth parameters of the plants, which are cultivated in boron toxicity conditions are negatively affected as a consequence of increases in osmotic

pressure that causes lack in water and destroy ions equilibration (Gossett et al., 1996). In this study, boron toxicity significantly decreased the shoots and roots growth of lentil and barley. The reduction in growth that resulted from B toxicity was significantly affected by changing nitrogen sources. The relative growth rate % was found to be minimal ($p < 0.05$) in NO_3^- fed plants in all shoot and root tissues and there was the lowest decrease in all tissues of NH_4^+ fed plants. Growth reduction under B toxic conditions is well documented in tomato (Cervilla et al., 2007), apple rootstocks (Mouhtaridou et al., 2004), barley (Karabal et al., 2003) and maize (Gunes and Alpaslan, 2000). Molassiotis et al. (2006) carried out increase in dry weight of apple roots when 0.5 mM boron was added. Conversely, Karabal et al. (2003) reported the decrease in fresh and dry weights of leaf and root of barley at high boron concentrations (10.0 mM). Jalloh et al. (2009) studied how the Cd toxicity affects the productivity of rice plant under the presence of certain nitrogen sources such as NH_4^+ , NO_3^- and urea. The group reported that when NH_4^+ was used, it gave the highest productivity. Britto and Kronzucker, (2002) obtained better grain yield, in barley cultivation, from NH_4^+ against to NO_3^- at their low concentrations. Similarly, Boukcim et al. (2001) reported that NH_4^+ -used *Cedrus atlantica* plant showed higher fresh weight than NO_3^- ones showed.

High concentrations of B may cause reductions in chlorophyll contents in plants. A typical symptom of B toxicity is characterized by the presence of chlorosis and necrosis, often at the margins and tips of older leaves (Nable et al., 1997). The results of this study indicated that total chlorophyll content significantly decreased when plants were exposed to boron stress and the decrease was more pronounced in NO_3^- and urea treated lentil. Similar results were reported that total chlorophyll content

Table 1. The effect of boron treatment (5 and 10 mM H₃BO₃) and different nitrogen sources (KNO₃, NH₄Cl and urea) on MDA, H₂O₂ and proline contents in shoots and roots of lentil and barley on day 7.

Shoot	MDA (μmol/gFW)		Proline (μmol/gFW)		H ₂ O ₂ (μmol/gFW)	
	Lentil	Barley	Lentil	Barley	Lentil	Barley
NO ₃ (Control)	48.19±1.76	43.41±1.32	27.94±2.48	23.04±1.51	10.9±0.54	8.79±0.32
NO ₃ +5B	57.31±0.66	49.27±0.65	33.72±2.67	27.14±1.30	14.24±0.50	11.26±0.70
NO ₃ +10B	61.07±1.61	54.15±1.25	40.26±2.32	35.01±1.13	17.85±0.48	14.06±0.54
NH ₄ (Control)	30.86±1.35	24.45±0.75	20.34±1.98	17.56±1.71	9.26±0.38	7.37±0.28
NH ₄ +5B	35.33±1.18	27.17±0.68	23.17±3.11	18.54±1.60	11.15±0.52	8.56±0.36
NH ₄ +10B	37.57±1.06	29.17±0.64	27.04±3.65	21.1±1.36	14.14±0.28	10.04±0.38
Üre(Control)	35.56±0.85	30.77±1.05	25.65±5.89	20.23±1.91	10.53±0.34	8.86±0.26
Üre+5B	43.23±1.62	36.78±1.15	30.14±4.45	23.12±1.83	13.45±0.42	11.05±0.40
Üre+10B	49.43±1.81	42.03±0.98	35.89±4.01	25.49±1.74	17.34±0.36	13.54±0.48
Root						
NO ₃ (Control)	53.45±1.92	47.79±1.41	28.86±2.57	25.14±1.62	17.09±0.68	15.48±0.44
NO ₃ +5B	64.27±1.80	54.99±1.27	35.59±3.03	30.62±1.59	23.36±0.58	20.87±0.49
NO ₃ +10B	68.71±1.69	64.26±1.20	44.53±4.56	37.77±1.50	28.55±0.49	24.97±1.68
NH ₄ (Control)	37.74±0.67	30.54±0.76	24.74±3.14	21.82±1.29	9.67±0.38	7.74±0.29
NH ₄ +5B	43.75±0.89	34.18±0.75	28.04±4.15	23.81±1.44	12.47±0.33	9.35±0.23
NH ₄ +10B	47.13±0.12	38.74±0.25	33.14±5.22	27.1±1.89	15.06±0.29	10.86±0.46
Üre(Control)	39.18±2.25	35.33±1.22	30.21±5.12	24.34±1.55	10.07±0.46	9.52±0.33
Üre+5B	48.25±1.95	43.19±1.35	34.86±5.05	27.86±1.61	13.55±0.44	12.04±0.38
Üre+10B	55.12±2.79	47.51±0.95	41.18±4.19	31.86±1.15	17.08±0.28	14.76±0.58

The results are the mean ±S.E. of three replicates.

decreased under B toxicity. A research on total chlorophyll content in *Fibro capsa japonica* (*Raphidophyceae*) was performed under changing nitrogen sources such as NH₄⁺, NO₃⁻ and urea by Cucchiari et al. (2008) and the highest total chlorophyll were determined in NH₄⁺ fed plants. Similar results were reported for *Catsetum fimbriatum* (Majerowicz et al., 2000).

Plants generate active oxygen species as a response against stress factors (Gosset et al., 1994). These generated-oxygen species destroyed cell membranes via lipid peroxidation. As a consequence of this situation, the cell membrane loses its semi permeable feature for ion uptake. Malondialdehyde (MDA) is the product of dissociation of unsaturated fatty acids of biomembrane. Proline has become one of the most attractive compounds in terms of plant stress physiology. The releases of this compound occurred under salt and water stresses and proline has a prominent role to trigger defense mechanism of the plants against the stress. Many plants accumulate non-harmful proline as an osmolyte under stress condition (Singh et al., 2000). Our results further indicated that MDA, proline and H₂O₂ contents were increased in both tissues of lentil and barley plants exposed to 5 and 10 mM B (Table 2). The lowest increase were obtained in the boron-stressed NH₄⁺ fed barley groups (p < 0.01). Karabal et al. (2003) exposed barley to boron stress and showed MDA, proline

and H₂O₂ increases accompany with this stress. Mittler (2002) proposed that membrane damage might be caused by high levels of H₂O₂, which accelerates the Haber-Weiss reaction, resulting in OH⁻ formation and thus, lipid peroxidation. Many researchers reported that MDA concentration increased as a result of boron toxicity in tomato (Cervilla et al., 2007), grapevine (Gunes et al., 2006), onion (Inal and Tarakcioglu, 2001) and apple (Molassiotis et al., 2006). In addition, some researchers reported that under heavy metal stress, the concentration of MDA increased in *Vigna* seedling (Bhattacharya and Choudhuri, 1995), lentil (Mishra and Chudhuri, 1996) and *Phaseolus vulgaris* (Somashékaraiah et al., 1992).

Under stress conditions, many metabolic reactions produce active oxygen species (AOS). However, plants possess an efficient system for scavenging AOS, which protects them from destructive oxidative reaction (Gosset et al., 1994). As a part of this system, antioxidant enzymes play important roles in the defense mechanism. In this study, we found that the activities of SOD, LOX and GPX in plants exposed to boron stress were significantly increased. On the other hand, CAT and APX activities were different in both species under boron toxicity. Compared with the controls, there were significantly higher APX and CAT activities in both tissues of barley under boron stress. In contrast, the activities of APX and CAT significantly decreased in lentil shoots and

roots with increasing boron concentration when compared with their. Different response of antioxidant enzyme activities to boron toxicity has been reported. Molassiotis et al. (2006) reported increased SOD, LOX and CAT activities under B toxicity in apple rootstocks. In addition to this, Garcia et al. (2001) in tobacco leaves, Karabal et al. (2003) in barley and Güneş et al. (2006) in grapevine have shown increased SOD and CAT activity under B toxicity. Similarly, Dube et al. (2000) reported that excess boron increased CAT activity in sunflower leaves. Our results also demonstrated that boron toxicity mediated changes in the activities of antioxidant enzymes in a cultivar, nitrogen sources and tissue dependent manner. The activities of the enzymes of the antioxidant system in plants under stress are usually regarded as an indicator of the tolerance of the genotypes against stress condition. Thus, in this study we intend to evaluate the antioxidant enzyme activity of tolerant and sensitive cultivars under boron toxicity stress, in order to evaluate the possible involvement of this system in tolerance mechanism to boron toxicity. It has been demonstrated that stress tolerant genotypes exhibited higher antioxidant enzyme activities (Sairam, 2002). In our experiments, lentil (sensitive) and barley (tolerant Yau, 2002) exhibited similar behaviour in terms of enhanced root and shoot SOD, LOX and GPX activities. However, there were differences in CAT and APX activities among the sensitive and tolerant cultivars. Interestingly, SOD, LOX and GPX activities of lentil are higher than barley. The CAT and APX activities in shoots and roots of lentil plants were higher in ammonium-fed plants in comparison to nitrate and urea fed plants. Jalloh et al. (2009) examined cadmium toxicity on rice under changing nitrogen sources medium. According to the study, SOD and GPX activities increased when NH_4^+ and urea were used as nitrogen sources. Similarly, Chao et al. (2008) reported increases in APX, GPX and CAT activities of *Vallisneria natans* with NH_4^+ , but SOD activity began to decrease over 20 to 28 mM NH_4^+ concentration. Gosset et al. (1994) reported that salt stress in *Gossypium hirsutum* L. caused increase in SOD and GPX activities, while CAT and APX decreased.

It can be concluded from this study that the oxidative damage and yield reduction of lentil and barley plants exposed to boron toxicity are dependent on N form and in general, the plants supplied with NH_4^+ have less oxidative damage and yield reduction caused by boron stress in comparison with the plants supplied with NO_3^- and urea. These results indicated the possibility of alleviating boron stress through improving fertilization.

REFERENCES

- Aebi H (1984). Catalase *In vitro*. *Methods Enzymol.*, 105: 121–126.
- Axelrod B, Cheesebrough TM, Laakso S (1981). Lipoxygenase from soybeans. EC 1.13.11.12 linoleate: oxygen oxidoreductase. *Methods. Enzymol.*, 71: 441–451.
- Bates L (1973). Rapid determination in free proline of water stress studies. *Plant Soil*, 39: 205.
- Beyer WF, Fridovich I (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal. Biochem.*, 161: 559–566.
- Bhattacharya M, Choudhuri MA (1995). Heavy metal (Pb^{2+} and Cd^{2+}) stress induced damages in *Vigna* seedlings and possible involvement of phytochelatin-like substances in mitigation of heavy metal stress. *Indian J. Exp. Biol.*, 33: 236–238.
- Bonilla I, Garcia-Gonzalez M, Mateo P (1990). Boron requirement in cyanobacteria. Its possible role in the early evolution of photosynthetic organisms. *Plant Physiol.*, 94: 1554–1560.
- Boukcim H, Pages L, Plassard C, Mousain D (2001). Root System Architecture and Receptivity to Mycorrhizal Infection in Seedlings of *Cedrus Atlantica* as Affected by Nitrogen Source and Concentration. *Tree Physiol.*, 21: 109–115.
- Britto DT, Kronzucker HJ (2002). NH_4^+ Toxicity in Higher Plants: a Critical Review. *J Plant. Physiol.*, 159: 567–584.
- Cervilla LM, Blasco BA, Rios JJ, Romero L, Ruiz JM (2007). Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to boron toxicity. *Ann. Bot.*, 100: 747–756.
- Chao W, Song HZ, Pei FW, Jun H, Wei L, Wen JZ (2008). Metabolic Adaptations to Ammonia-induced Oxidative Stress in Leaves of the Submerged Macrophyte *Vallisneria Natans* (Lour.) Hara. *Aquat. Toxicol.*, 87: 88–98.
- Cucchiari E, Franca G, Antonella P, Cecilia T, Rossella P (2008). Effect of Salinity, Temperature, Organic and Inorganic Nutrients on Growth of Cultured *Fibrocapsa Japonica* (*Raphidophyceae*) from the Northern Adriatic Sea. *Harmful Algae*, 7: 405–414.
- Dat J, Vandenabeele S, Vranova E, Van Montagu M, Inze D, Van Breusegem F (2000). Dual Action of the Active Oxygen Species During Plant Stress Responses. *Cell. Mol. Life. Sci.*, 57: 779–795.
- Dordas C, Brown PH (2000) Permeability of boric acid across lipid bilayers and factors affecting it. *J. Membr. Biol.*, 75: 95–105.
- Dube BK, Sinha P, Chatterjee C (2000). Boron stress affects metabolism and seed quality of sunflower. *Trop. Agric.*, 77: 89–92.
- Gossett DR, Millhollon EP, Lucas MC (1994). Antioxidant Response to NaCl Stress in Salt-Tolerant and Salt-Sensitive Cultivars of Cotton. *Crop. Sci.*, 34: 706–714.
- Gunes A, Alpaslan M (2000). Boron uptake and toxicity in maize genotypes in relation to boron and phosphorus supply. *J. Plant. Nutr.*, 23: 541–550.
- Gunes A, Soylemezoglu G, Inal A, Bagci EG, Coban S, Sahin O (2006). Antioxidant and stomatal response of grapevine (*Vitis vinifera* L.) to boron toxicity. *Sci. Hort.*, 110: 279–284.
- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. *Arch. Biochem. Biophys.*, 125: 189–198.
- Inal A, Tarakcioglu C (2001). Effects of Nitrogen Forms on the Growth, Nitrate Accumulation, Membran Permeability, and Use Efficiency of Hydroponically Grown Bunch Onion (*Allium cepa* L. Var. Radar) Boron Deficiency and Toxicity. *J. Plant Nutr.*, 24: 1521–1534.
- Jalloh MA, Jinghong C, Fanrong Z, Guoping Z (2009). Effect of Different N Fertilizer Forms on Antioxidant Capacity and Grain Yield of Rice Growing under Cd Stress. *J. Hazardous. Mater.*, 162: 1081–1085.
- Jana S, Choudhuri MA (1981). Glycolate metabolism of three submerged aquatic angiosperms during aging. *Aquat. Bot.*, 12: 345–354.
- Karabal E, Yucel M, Oktem HA (2003). Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. *Plant Sci.*, 164: 925–933.
- Lichtenthaler HK, Wellburn AR (1983). Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochem. Soc. Trans.*, 11: 591–592.
- Majerowicz N, Nievola CC, Susuki RM, Kerbauy GB (2000). Growth and nitrogen metabolism of *Catasetum fimbriatum* plants (Orchidaceae) grown with different nitrogen sources. *Environ. Exp. Bot.*, 44: 195–206.
- Migge A, Becker TW (1996). In tobacco leaves, the genes encoding the nitrate-reducing or the ammonium-assimilating enzymes are regulated differently by external nitrogen sources. *Plant. Physiol. Biochem.*, 34: 665–671.
- Mishra A, Choudhuri MA (1996). Possible implications of heavy metals (Pb^{2+} and Hg^{2+}) in the free radical mediated membrane damage in

- two rice cultivars. *Indian. J. Plant. Physiol.*, 1: 40–43.
- Mitler R (2002). Oxidative stress, antioxidants and stress tolerance, *Trends Plant. Sci.*, 7: 405–410.
- Molassiotis A, Sotiropoulos T, Tanou G, Diamantidis G, Therios I (2006). Boron-induced oxidative damage and antioxidant and nucleolytic responses in shoot tips culture of the apple rootstock EM 9 (*Malus domestica* Borkh). *Environ. Exp. Bot.*, 55: 54–62.
- Mouhtaridou GN, Sotiropoulos TE, Dimassi KN, Therios IN (2004). Effects of boron on growth, and chlorophyll and mineral contents of shoots of the apple rootstock mm 106 cultured *in vitro*. *Biol. Plant.*, 48: 617–619.
- Nable RO, Banuelos GS, Paul JG (1997). Boron toxicity, *Plant. Soil.*, 198: 181–198.
- Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell. Physiol.*, 22: 867–880.
- Sairam RK, Srivastava GC (2002). Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress, *Plant Sci.*, 162: 897–904.
- Singh SK, Sharma HC, Goswami AM (2000). Datta SP and Singh SP, *In vitro* Growth and Leaf Composition of Grapevine Cultivars as Affected by Sodium Chloride, *Biol. Plant.*, 43: 283–286.
- Smirnov N (1993). The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol.*, 125: 27–58.
- Somashekaraiah BV, Padmaja K, Prasad ARK (1992). Phytotoxicity of Cadmium Ions on Germinating Seedlings of Mung Bean (*Phaseolus vulgaris*): Involvement of Lipid Peroxides in Chlorophyll Degradation. *Physiol. Plant.*, 85: 85–89.
- Ullrich WR (2002). Salinity and nitrogen nutrition. In: Lañuchli A, Luñttge U (Eds), *Salinity: Environment Plants Molecules*. Kluwer Academic Publishers, the Netherlands, pp. 229–248.
- Weast RC (1978). *Handbook of Chemistry and Physics*. 58th ed. CRC Press, Cleveland, OH.
- Yau SK, Tahir M (1996). Variation in boron-toxicity tolerance causes G × E interaction in yield. p. 138. Abstracts of poster sessions, 2nd Int. Crop Science Congr., New Delhi, India. pp. 17–24.
- Yau SK (2002a). European versus West Asian and North African winter barleys in tolerance to boron toxicity. *Euphytica*, 123: 307–314.