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*Full Length Article*

# Interactive Effect of Boron and Salinity on Growth, Physiological and Biochemical Attributes of Wheat (*Triticum aestivum*)

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## Abstract

A pot experiment was conducted to investigate the interactive effect of salinity (125 mM) and varying concentrations of boron (2, 4 and 6 mM) on the growth and biochemical attributes of wheat. Results showed that application of 4 and 6 mM boron (B) along with 125 mM NaCl significantly declined plant biomass and leaf length. The concentration of B in plant leaves was significantly lower in treatments where salinity and B were applied together as compared to individual applications of 2, 4 and 6 mM B. The membrane permeability and protein concentration were significantly increased by the combined application of NaCl and B, whereas the chlorophyll pigments were not influenced. The phenolic compounds and ascorbic acid concentrations were reduced with the individual applied 6 mM B and when combined with application of 125 mM NaCl + 6 mM B. The concentration of Malondialdehyde (MDA) gradually increased by increasing B application and maximum was at the highest level of B and NaCl stress. It is concluded that salinity worsens the deteriorating effect of boron toxicity on wheat growth. 

**Keywords:** Salt affected soils; Boron; Biochemical activities; Wheat

## Introduction

Soil salinity influences availability of boron (B) to crop plants. In salt-affected soils, B toxicity is a continuing problem even after reclamation of salinity. Therefore, the response of crops to B and salinity remains focus of study in salt-affected soils. Generally salinity reduces the B uptake, however, contradictory results revealed that salinity can decrease or enhance the B toxicity in wheat (Wimmer *et al*., 2001). Under salinity, B worsens the activity of various membrane components and functions of certain aquaporin isoforms which influence the water uptake and its transport (Martínez-Ballesta *et al*., 2008). The salinity stress and B interactively affects the germination, shoot and root length, shoot root dry matter and increase the membrane permeability (Ismail, 2004; Molassiotis *et al*., 2006). At higher B concentration in growth medium, B enters plant tissues and alters the cell system and interferes the different physiological processes leading to drastic decrease in net yield of crops (Gupta, 1982; Nable *et al*., 1997).

Growth and biochemical reactions in sunflower are negatively affected by foliar application of B under saline conditions (Jabeen and Ahmad, 2011). According to Wimmer *et al*. (2003), salinity and B toxicity have interactive and combined effects on uptake of B, water and B partitioning within the plant. Bingham *et al*. (1987) found no significant interaction between salinity and B toxicity in wheat grown at different B and salinity levels. Smith *et al*. (2013) investigated interactive effect of salinity, B and pH on the growth of Broccoli and found significant reduction in yield by both salinity and B application, however the effect was more pronounced at high pH. The reduction in shoot fresh mass and increase in total antioxidative capacity (TAC) and Luminol-converting peroxidase (LUPO) was more under combined stress of salinity and B than salinity alone (Masood *et al*., 2012).

A plenty of literature is available about effects of NaCl and B either combined or alone on various plant species. However, less research has been done on interactive effect of NaCl and B on growth, and biochemical attributes in salt sensitive genotypes of wheat. It was hypothesized that



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salinity enhances the toxic effects of B on growth rate and biochemical characteristics of wheat plants.

## Materials and Methods

A pot experiment was conducted under greenhouse conditions at experimental station of Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut Egypt. The experiment was laid out as complete randomized design (CRD) having eight treatments and three replications on a wheat (*Triticum aestivum*) cultivar (Giza 168). The treatments included: control (without NaCl), 125 mM NaCl, 2 mM B, 2 mM B+ NaCl, 4 mM B, 4 mM B + NaCl, 6 mM B and 6 mM B + NaCl. The external nutrients applied were as, $NH_4NO_3$ (0.380 g $kg^{-1}$ soil); Superphosphate (1.910 g $kg^{-1}$ soil); $MgCO_3$ (0.133 g $kg^{-1}$ soil) and $H_3BO_3$ (0.003 g $kg^{-1}$ soil).

Salinity and B levels were maintained in the saturated soil extract by adding and mixing the calculated amount of boric acid (17.48% B) and sodium chloride in soils. Twenty-five seeds were grown in normal clay soil in plastic pots (30 cm in diameter) containing 3 kg soil. Twenty plants (after seeding) were allowed to grow in the presence of various treatments for 28 days and then harvested. Data was collected immediately on fresh plant biomass and leaf length after harvest. The whole shoots and roots were dried at 80°C for 24 h, after which shoot and root dry weights were collected.

### Determination of Physiological and Biochemical Parameters

For the determination of cations, about 200 mg dried wheat-leaves were dry-ashed at 550°C overnight in muffle furnace. After cooling, samples were digested in 5 M $HNO_3$ by heating prior to boiling. The concentrations of sodium (Na), potassium (K) and calcium (Ca) in filtrates were determined using flame-photometer (model M7D). For determination of boron, ground plant materials were taken in porcelain crucible. After dry ashing at 550°C for 6 h, sulphuric acid was added in the crucibles and filtrates were taken. The subsequent measurement of B concentration in filtrates was done colorimetry using Azomethine-H (Bingham, 1982). The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in leaves were estimated using UV-VIS spectrophotometer (ZWOCO 5060806) following the methods of Lichtenthaler (1987). Free proline content was determined according to Bates *et al.* (1973) using calibration curve of proline and expressed as mg $g^{-1}$ dry weight (D. wt).

Total protein content was determined according to the method of Lowry *et al.* (1951). Electrical conductivity of wheat was assessed in order to study the degree of membrane injury caused by salinity and boron. The cell membrane stability was determined using the method prescribed by Premachandra *et al.* (1992) with minor modifications.

The lipid peroxidation was determined by measuring malondialdehyde (MDA) formation using the thiobarbituric acid reaction as described by Rao and Sresty (2000) with some modifications. The concentration of MDA was calculated by using an extinction coefficient (155 $mM^{-1}$ $cm^{-1}$) and the results expressed as µmol $g^{-1}$ F. wt. The concentration of total phenolics was determined according to Kofalvi and Nassuth (1995). For phenolics determination, fresh leaves (0.5 gm) were extracted in 50% methanol (1: 1 v/v) for 90 min at 80°C. The extract was centrifuged at 14000 rpm for 15 min. Total phenolics were determined in the supernatant using the Folin-Ciocalteu's phenol reagent (Kofalvi and Nassuth, 1995). The concentration of ascorbic acid in the leaves was determined according to methods of Jagota and Dani (1982). The measurements were made taking the absorbance readings of the blue color developed at 760 nm using spectrophotometer.

### Statistical Analyses

The analysis of variance (ANOVA) and post hoc Tukey-HSD were performed for statistical comparison of treatments using statistical software package SPSS version 18.0.

## Results

### Shoot, Root Fresh and Dry Biomass

The results showed significant decrease in shoot and root fresh biomass as compared to control treatment when boron and salinity treatments were applied in combination at higher application rates (4 mM B + NaCl and 6 mM B + NaCl). However, at lower rates of B and NaCl, no significant effect was observed (Fig. 1). The root fresh biomass was significantly reduced in both treatments (125 mM NaCl and 6 mM B + NaCl) as compared to the control.

In comparison to control, B stress alone at all levels significantly reduced the shoot dry biomass (Fig. 1B). However, shoot dry biomass among different B stress treatments was not significantly different. Salt stress either alone or in combination with different B stress levels also reduced the shoot dry biomass in comparison to control and B alone treatments. Maximum reduction in shoot dry biomass was observed where plants were treated with 6 mM B + NaCl (Fig. 1B). On the other hand, B stress treatments did not influence the root dry biomass as compared to that of control. Boron applications of 2 and 4 mM significantly produced greater root dry biomass than 6 mM B treatment. Whereas, salt treatment either alone or in combination with 4 and 6 mM B significantly decreased the root dry biomass as compared to control.

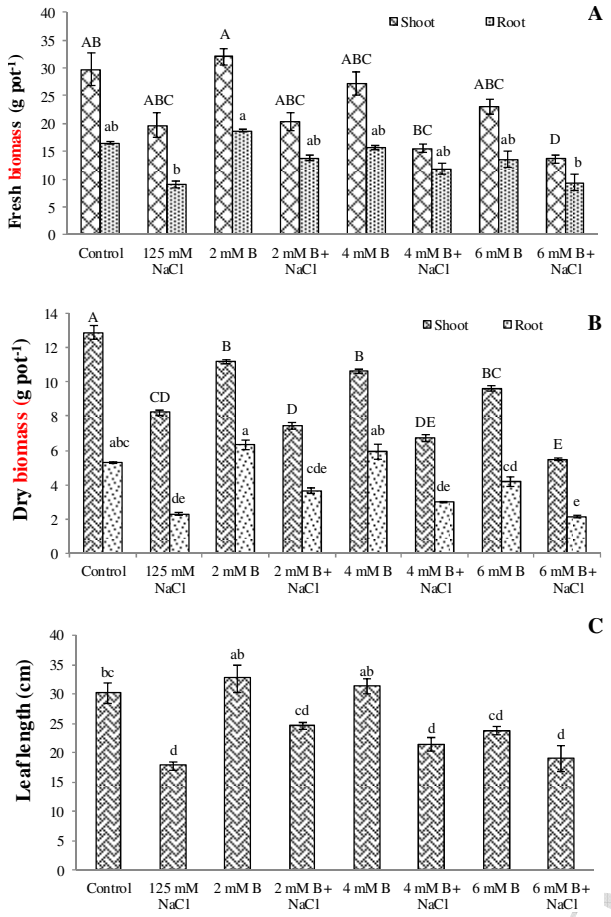


**Fig. 1:** Individual and combined effects of salinity (125 mM NaCl) and boron stress (2, 4, 6 mM $H_3BO_3$) on shoot & root fresh mass (A), shoot and root dry mass (B) and leaf length (C) of wheat

Bars represent means ±S.E. of three replicates and different letters indicate significant differences among treatments. Significant differences among treatments for shoot fresh and dry mass are shown with capital letters

## Leaf Length

Application of 2 and 4 mM B did not affect the leaf length, while 6 mM slightly reduced it as compared to control (Fig. 1C). Significantly greater leaf length was produced by 2 and 4 mM B than 6 mM B. Similarly, combined application of salt stress with 4 and 6 mM B significantly produced less leaf length than control. However, no significant differences were observed among salinity alone and combined application of salinity with different B stress treatments.

## Interactive Effects of Boron Stress and Salinity on Cation Concentrations in Wheat Leaves

The concentration of boron in plant leaves significantly increased by increasing boron supply in nutrient medium (Table 1). As compared to control, NaCl did not significantly affect B concentration. Addition of 125 mM NaCl to the nutrient medium containing 2, 4 or 6 mM B significantly reduced the leaf B concentrations as compared to individual applications of B, respectively. Salt stress significantly increased the leaf $Na^+$ concentration (Table 1).

**Table 1:** Individual and combined effects of salinity and boron stress on the leaf B, Na, K, and Ca concentrations of wheat

| Treatments[1] | B | $Na^+$ | $K^+$ | $Ca^{2+}$ |
|---|---|---|---|---|
| | (mg $kg^{-1}$ DM) | | | |
| Control | 18.36 f | 1.84 d | 212.29 a | 21.10 b |
| NaCl | 33.39 f | 13.63 c | 165.19 b | 23.29 b |
| 2 mM B | 190.34 cd | 1.37 d | 219.22 a | 30.34 ab |
| 2 mM B + 125 mM NaCl | 120.01 e | 29.86 a | 207.05 ab | 25.39 b |
| 4 mM B | 253.39 b | 1.77 d | 179.76 b | 35.10 a |
| 4 mM B + 125 mM NaCl | 160.22 d | 21.97 b | 189.00 ab | 26.52 ab |
| 6 mM B | 323.91 a | 1.29 d | 165.87 b | 23.86 b |
| 6 mM B + 125 mM NaCl | 196.53 b | 17.83 bc | 184.83 ab | 21.24 b |

The different letters within each column show significant differences among treatments at P≤0.05

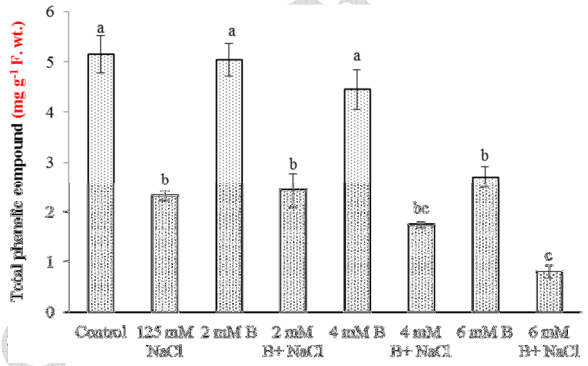


**Fig. 2:** Individual and combined effects of salinity (125 mM NaCl) and boron stress (2, 4, 6 mM $H_3BO_3$) on total phenolic compounds in shoot

Bars represent means ±S.E. of three replicates and letters indicate significant differences among treatments

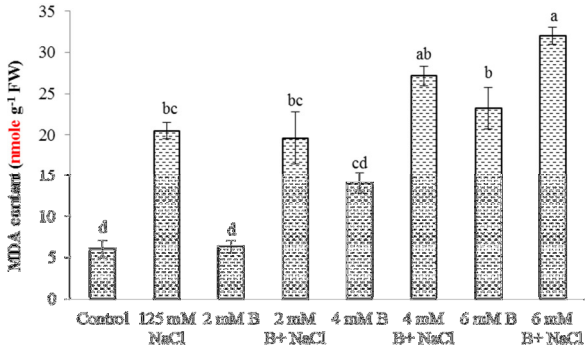


**Fig. 3:** Individual and combined effects of salinity (125 mM NaCl) and boron stress (2, 4, 6 mM $H_3BO_3$) on MDA contents

Bars represent means ±S.E. of three replicates and different letters indicate significant differences among treatments

**Table 2:** Individual and combined effects of salinity and boron stress on leaf membrane permeability, total protein, total pigments, carotenoids, chlorophyll a, and chlorophyll b content in wheat.

| Treatments[1] | Membrane permeability (EC %) | Protein content (mg $g^{-1}$ DW) | Total pigments (mg $g^{-1}$ FM) | Carotenoids (mg $g^{-1}$ FM) | Chl a (mg $g^{-1}$ FM) | Chl b (mg $g^{-1}$ FM) |
|---|---|---|---|---|---|---|
| Control | 21.10 c | 5.26 bc | 26.20 bc | 4.93 bc | 15.72 a | 5.73 abc |
| NaCl | 31.07 bc | 11.50 a | 24.00 bc | 5.22 bc | 13.42 ab | 5.55 bc |
| 2 mM B | 21.11 c | 6.56 bc | 32.21 a | 7.81 a | 16.22 a | 8.34 a |
| 2 mM B + 125 mM NaCl | 35.00 b | 13.29 a | 28.27 ab | 6.21 ab | 14.80 ab | 6.95 ab |
| 4 mM B | 21.66 c | 4.99 c | 26.86 abc | 6.12 ab | 14.02 ab | 6.74 ab |
| 4 mM B + 125 mM NaCl | 41.16 ab | 11.53 a | 21.24 cd | 4.86 bc | 10.90 bc | 5.32 bc |
| 6 mM B | 20.81 c | 3.75 c | 23.23 bc | 4.81 bc | 13.19 ab | 5.25 b |
| 6 mM B + 125 mM NaCl | 47.95 a | 7.98 b | 15.64 d | 3.64 c | 8.42 c | 3.61 c |

[2]Letters within each column show significant differences among treatments at P≤0.05

**Table 3:** Correlation among various parameters determined during interactive study of B and salt in wheat

| | B | $Na^+$ | $K^+$ | $Ca^{2+}$ | Membrane permeability | Protein cont. | Chl a | Chl b | Caretenoids | Total pigments |
|---|---|---|---|---|---|---|---|---|---|---|
| B | 1.000 | -0.27 | -0.259 | 0.356 | -0.127 | -0.476* | -0.206 | 0.008 | 0.038 | -0.096 |
| $Na^+$ | -0.27 | 1.000 | 0.054 | -0.245 | 0.756** | 0.880** | -0.382 | -0.201 | -0.225 | -0.307 |
| $K^+$ | -0.259 | 0.054 | 1.000 | 0.062 | -0.145 | 0.080 | 0.392 | 0.442* | 0.395 | 0.461* |
| $Ca^{2+}$ | 0.356 | -0.245 | 0.062 | 1.000 | -0.281 | -0.159 | 0.195 | 0.604** | 0.587** | 0.447* |
| Membrane permeability | -0.127 | 0.756** | -0.145 | -0.281 | 1.000 | 0.595** | -.726** | -.549** | -0.537** | -0.698** |
| Protein cont. | -0.476* | .880** | 0.080 | -0.159 | 0.595** | 1.000 | -0.202 | -0.017 | -0.003 | -0.102 |
| Chl a | -0.206 | -0.382 | 0.392 | 0.195 | -0.726** | -0.202 | 1.000 | .633** | 0.684** | 0.916** |
| Chl b | 0.008 | -0.201 | 0.442* | 0.604** | -0.549** | -0.017 | 0.633** | 1.000 | 0.837** | 0.874** |
| Caretenoids | 0.038 | -0.225 | 0.395 | 0.587** | -0.537** | -0.003 | 0.684** | 0.837** | 1.000 | 0.886** |
| Total pigments | -0.096 | -0.307 | 0.461* | 0.447* | -0.698** | -0.102 | 0.916** | 0.874** | 0.886** | 1.000 |

[1]* Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed)

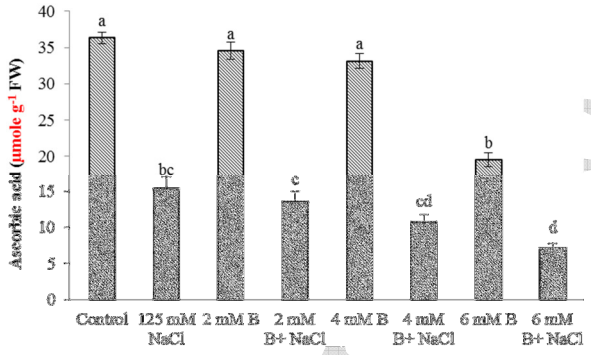


**Fig. 4:** Individual and combined effects of salinity (125 mM NaCl) and boron stress (2, 4, 6 mM $H_3BO_3$) on ascorbic acid concentration

Bars represent means ±S.E. of three replicates and letters indicate significant differences among treatments

However, B supply at different levels did not influence the $Na^+$ concentration. While application of 2 and 4 mM B to salt-stressed plants significantly increased the $Na^+$ concentration as compared to NaCl alone. Application of 6 mM B to salt-stressed plants did not further increase the Na concentration as compared to NaCl alone. Boron supply at high rates (4 and 6 mM B) significantly reduced the $K^+$ concentration, while 6 mM B did not influence the $K^+$ concentration as compared to control plants (Table 1). Individual application of NaCl reduced the $K^+$ concentration, however when supplied in combination with different B levels, it did not affect the $K^+$ concentration in comparison to control. The concentration of $Ca^{2+}$ in leaves was significantly increased with the application of 4 mM B and rest of the treatments did not pose significant influence on the $Ca^{2+}$ concentration.

**Interactive Effects of Boron Stress and Salinity on Membrane Permeability, Protein, Total Pigments, Carotenoids, Chl *a* and Chl *b***

The individual applications of NaCl and B at all levels did not significantly influence the membrane permeability (Table 2), while the combined applications of B and NaCl significantly increased the membrane permeability with increasing B rates. Application of B did not influence leaf protein contents; however, salt stress alone or in combination with B significantly increased the leaf protein contents as compared to control plants (Table 2). Application of 2 mM B significantly increased the total pigments and carotenoid contents as compared of control, while application of higher levels of B did not influence the total pigments and carotenoid contents (Table 2). Salt stress alone or in combination with 2 and 4 mM B did not affect the total pigments and carotenoids. However, combined application of NaCl and 6 mM B significantly reduced the total pigments and carotenoid contents. Both individual applications of salinity (125 mM NaCl) and B levels (2, 4 and 6 mM B) did not affect the contents of Chl *a* and Chl *b*. Combined application of NaCl and 6 mM as compared to control significantly reduced the Chl *a* contents (Table 2).

**Antioxidant Defense System**

The phenolic contents in wheat leaves were not affected by application of 2 and 4 mM B as compared to control; however, 6 mM B significantly reduced the phenolic contents (Fig. 2). On the other hand, application of 125 mM NaCl alone or in combination with different B levels significantly reduced the total phenolic contents in wheat leaves. The maximum reduction in total phenolic contents was observed in plants supplied with 125 mM NaCl + 6 mM B (Fig. 2).

Malondialdehyde (MDA) is a decomposition product of lipids and is often utilized as biomarker for lipid peroxidation. Our results showed that MDA concentrations (lipid peroxidase) were significantly increased by 6 mM B (Fig. 2). Salt stress alone or in combination with high B significantly enhanced the leaf MDA concentration (Fig. 2). The maximum increase was found in combined application of 6 mM B + NaCl. In comparison to salt stress alone, MDA concentration increased only under application of 6 mM B + NaCl.

Application of 2 and 4 mM B did not affect the leaf ascorbic acid concentrations as compared to control treatment (Fig. 4). However, 6 mM B significantly reduced the concentration of ascorbic acid. On the other hand, salt stress either alone or in combination with B decreased the ascorbic acid concentration by more than two folds. The combined effect of salinity and boron stress on ascorbic acid concentration was only prominent at 6 mM B + NaCl.

**Correlation Studies**

Leaf B concentration had a significant negative correlation with total protein contents (Table 3). The $Na^+$ concentration had significant positive correlation with membrane permeability and protein content. Membrane permeability had significant negative correlations with chlorophyll *a*, *b*, carotenoids and total pigments.

## Discussion

In present study, a significant growth reduction of wheat cultivar (cv. Giza 168) was observed by boron toxicity, salinity and their interaction. Plant growth was severely reduced by interactive effect of salinity and high level of B toxicity as compared to their individual effect. In different studies, both salinity and B toxicity showed significant reduction in germination of maize and sorghum (Ismail, 2004). It has been reported that interaction of salinity and B toxicity decreased growth and yield of wheat (Grieve and Poss, 2000). Boron toxicity causes decrease of (i) leaf area, i.e., reduced expansion of meristematic tissues, (ii) supply of photosynthates to growing parts (Nable *et al*., 1997) and (iii) photosynthesis with increasing necrosis of mature plant parts (Reid *et al*., 2004).

The significant decrease in B concentration at saline conditions might be due to either increased pH of soil by addition of NaCl or due to salinity induced stomatal resistance because B uptake is a passive process and influenced by transpiration rate and reduced uptake and translocation in plant (Eraslan *et al*., 2007). This can be regarded as mechanism of reducing the severity of B toxicity by decreasing B absorption in shoot and stem (Rozema *et al*., 1992; Grattan *et al*., 1997). However, accumulation of B differs with growth conditions and crop, e.g., maize and barley did not show interactive response of salt stress and B (Shani and Hanks, 1993) and another study showed that B uptake was increased in *Zea mays* L. (Bastías *et al.*, 2004). Increased concentration of $Na^+$ and decreased concentration of $K^+$ (Table 1), as observed in our study are also well known (Al-Hakimi and Hamada, 2001). The salinity and $K^+$ have strong negative correlation. The increase of salinity reduces the $K^+$ concentration in shoot because there is competition of $Na^+$ and $K^+$ under salinity stress (Läuchli and Grattan, 2007). Application of B did not affect $K^+$ and $Ca^{2+}$ concentration significantly that might be due to no competition between these due to their specific pathways for uptake by plants (Zia *et al*., 2003; Wang and Wu, 2013).

Membrane stability index is an indicator of salt tolerance in plants and its values are relatively higher in salt tolerant as compared to salt sensitive wheat cultivars (Sairam *et al*., 2002), but membrane permeability was significantly higher in tomato and cucumber under salinity stress along B toxicity (Alpaslan and Gunes, 2001) as found in wheat of present study (Table 2). It may be due to B role as structural component of plasma membrane, forms complexes with sugar, glycoprotein and phospholipid and influences different membrane associated processes (Brown *et al*., 2002; Bonilla *et al*., 2004) and stabilizes membrane structure (Cakmak *et al*., 1995). Boron toxicity and NaCl both influence antioxidant activities that scavenge reactive oxidative species (ROS) and metabolites. Under ionic stress, B toxicity increases production of ROS (Cervilla *et al.*, 2007). Protein concentration under salinity was increased that may be due to reduced dilution factor resulting after reduced growth. Boron toxicity have little effect on total phenolics only at higher B levels, otherwise the effect was not significant (Fig. 2).

Salinity stress significantly increased concentration of MDA in present study. Abiotic stresses especially salinity causes membrane damage through membrane lipid peroxidation (Mishra and Choudhuri, 1999). Our findings show significant correlation between membrane permeability and MDA. Mittler (2002) measured $H_2O_2$ and MDA concentrations in leaves under oxidative stress. $H_2O_2$ caused membrane damage that fasten the Haber-Weiss reaction, by production of hydroxyl radical ($OH^-$) and lipid peroxidation. MDA contents were significantly increased under B toxicity in this study (Fig. 3); however contradictory reports are available for this. For instance,

Karabal *et al*. (2003) reported that there is no relationship between lipid peroxidation and $H_2O_2$ concentration under B toxicity in barley but in other study both $H_2O_2$ and MDA concentrations were increased in grape (Gunes *et al*., 2006) and apple rootstock under high B toxicity (Molassiotis *et al*., 2006). Though, $H_2O_2$ concentration was increased under combined B toxicity and salinity stress correlates significantly with SOD activity, not with peroxidase, as dismutation of superoxide to $H_2O_2$. More often, peroxidases might produce $H_2O_2$ by paradoxically (Sairam *et al*., 2005).

Ascorbic acid acts as a primary substrate for neutralization and detoxification of ROS species (superoxide radicals and singlet oxygen) in the cyclic pathway because it is a small water-soluble antioxidant molecule (Noctor and Foyer, 1998). In our findings, significant decrease of ascorbic acid was observed in combined NaCl and B application; individually-applied B toxicity did not affect much at lower level, however, salinity has rapid and significant affects. A destructive effect of Na on enzyme activity reduces the ascorbic acid production and photosynthesis is directly affected by its deficiency (Smirnoff, 2000).

## Conclusion

Present study conclude that B toxicity did not enhance the injurious effect of salt stress as wheat growth was not reduced by B application under salinity; however salinity further reduced the plant growth under B toxicity. Although B concentration in leaves was decreased under salinity, but increased MDA concentration and reduced ascorbic acid concentration in leaves were found due to B and NaCl. Furthermore, B and NaCl may have similar mode of metabolic deterioration in the plant tissue causing oxidative stress to the plants.

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