



Boron Induces Seed Germination and Seedling Growth of *Hordeum Vulgare* L. Under NaCl Stress

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ABSTRACT

Boron (B), an essential micronutrient, helps the plants to complete their life cycle successfully. Therefore, the present experiment was conducted to study (1) the role of B in seed germination and seedling growth, (2) the toxicity effect of B in seed germination and seedling growth and (3) the role of B in tolerance of barley (*Hordeum vulgare* L. var. 'Bakore') to NaCl stress. Under NaCl stress and non-stress conditions, application of high levels of B (100 μ M) decreased parameters of germination (G%, VI, GI and MGT), growth (RL, SL, RFW, SFW, RDW and SDW), except the accumulation of Pro and MDA in barley seedlings. Also, a fluorescence study reveals that production of ROS (H_2O_2 and $O_2^{\cdot-}$) and non-viable cells increased in roots of barley seedlings treated with NaCl and high dose of B. An alteration in anatomical structure of barley seedlings was observed with the application of NaCl and high dose of B. However, a low concentration of B (50 μ M) proved best and increased all germination and growth traits of barley seedlings by increasing further accumulation of Pro. Also, 50 μ M of B significantly increased the biosynthesis of photosynthetic pigments (Chl *a*, *b* and total Chl) and decreased formation of ROS and viable cells in roots. Therefore, concluded that sufficient dose of B could be beneficial for barley plant in improving the tolerance to NaCl stress.

Indexing terms/Keywords: Boron Toxicity; *Hordeum Vulgare* L.; Salinity; Germination; Reactive Oxygen Species

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INTRODUCTION

Like other essential nutrients, boron (B) is also a vital micronutrient for plants to complete their life cycle successfully. It plays a key role in cell wall formation by forming a crosslink between dimers of the pectin rhamnogalacturonan II which provides a stable and complex cell wall with decreased pore space (a low-molecular-mass pectic polysaccharide) (Matoh, 1997; O'Neill et al., 2004; Corrales et al., 2008). B, a unique essential micronutrient, has a very thin line between toxicity and deficiency which is based on a suitable concentration. A suitable concentration of B varies among plants species and even among cultivars of the same species (Siddiqui et al., 2013; Keren and Bingham, 1985). Deficient and toxic levels of B in soil lead to the morphological and physiological disorder in plants. Some countries (India, China and US) of world are suffering from the B deficiency, whereas arid and semiarid regions are affected by the B toxicity. The surface mining, fly ash and industrial chemicals are main source of elevated B (Nable et al., 1997). Area of some countries such as Chile, India, Peru, Israel, South Australia, North Africa, West Asia, Egypt, Iraq, Libya, Jordan, Morocco, Syria, Turkey, and the west coast of Malaysia are suffering from B toxicity (Yau and Ryan 2008). Like other abiotic stress, elevated levels of B in soil cause formation of reactive oxygen species [hydrogen peroxide (H_2O_2), superoxide ($O^{\cdot-}$) and hydroxyl ($OH^{\cdot-}$) radical], which cause oxidative damage by oxidizing lipids, proteins, and nucleic acids (Ardic et al., 2009; Cervilla et al., 2007). However, B in sufficient amount maintains cell membrane integrity and also improves the cellular defense mechanism in plants (Xuan et al., 2001; Siddiqui et al., 2013). However, the mechanisms involved in B tolerance and toxicity in plants are still poorly defined (Cervilla et al., 2007; Fitzpatrick and Reid 2009).

During the plant life cycle, the process of seed germination is an important event, which guarantees the better growth and survival of plants. The increased levels of B in the soil seize seed germination and inhibit cell wall expansion and photosynthetic pigment synthesis and also reduce lignin and suberin formation (Nable et al., 1997; Reid 2007). Foliar application of B increased growth yield of tomato plant (Harris and Puvanitha, 2018). A high percentage of abnormal seedlings of black gram and green gram was observed with 9 mg B kg^{-1} (Rerkasem et al., 1989) and in other experiment low germination percentage was recorded with less than 6 mg B kg^{-1} (Bell et al., 1989). Rerkasem et al., (1997) conducted a preliminary unfertilized river sand experiment on soybean and reported that application of 10, 14, 16 and 20 mg B kg^{-1} gave 98 to 100% germination but produced 26, 14, 5 and 7% abnormal seedlings, respectively. Farag and Fang (2014) applied 0, 10, 25, 50 & 100 mg B L^{-1} on watermelon cultivars and found that high levels of B had not significant effect on seed germination percentage, but increased mean germination time and germination index. The NLN-medium contained 162 μM improved microspore embryogenesis in four genotypes of *Brassica* species, but no significant effect on plant conversion from the regenerated embryo was observed (Mahasuk et al., 2017). The available literature reveals that the role of B in seed germination is not fully understood. Therefore, the present experiment aimed to study (1) role of B in seed germination and seedling growth, (2) toxicity effect of B in seed germination and seedling growth and (3) role of B in tolerance of wheat/barley plants to NaCl stress.

MATERIALS AND METHODS

Preparation of seeds and boron application

The experiment was performed under laboratory conditions using barley (*Hordeum vulgare* L. var. 'Bakore') obtained from a local market of Riyadh, Saudi Arabia. Sodium hypochlorite solution (10%) was used to sterilized selected healthy seeds. After 10 min of sterilization, seeds were rinsed properly with double-distilled water (DDW) before placing into Petri dish (Size 12 in) having a double layer of filter papers. One hundred fifty sterilized seeds were placed into each Petri Dish and all petri dishes were arranged in a simple randomized design with single factor and 4 replicates. Treatments of B were applied with and without NaCl as follows (1) 0 μM B + 0 mM NaCl (control), (2) 50 μM B + 0 mM NaCl, (3) 100 μM B + 0 mM NaCl, (4) 0 μM B + 100 mM NaCl, (5) 50 μM B + 100 mM NaCl and (6) 100 μM B + 100 mM NaCl. To avoid evaporation, each Petri Dish was sealed with paraffin tape after supplying treatments. Boric acid was used as a source of B. The petri dishes of all treatments were placed in an incubator at $28 \pm 3\text{ }^\circ\text{C}$.



We recorded germinated seeds number every day. To avoid contamination, after every 3 d, all treated seedlings were transferred into new sterile dishes having sterile filter papers soaked with same concentrations and volume of treatments. The potential of seed germination was assessed in terms of percent seed germination, mean germination time (MGT), germination index (GI) and vigour index (VI). At the end of the 2 week, length of root (RL) and shoot (SL), fresh weight of root (RFW) and shoot (SFW) and dry weight of root (RDW) and shoot (SDW) seedling⁻¹ were measured.

Determination of growth characteristics

The seed germination rate was recorded every day from 2 to 14 d. Seeds were considered as germinated when their radicle showed at least 2-mm length.

Germination percentage (GR%) was calculated with the suitable formula:

$$GR(\%) = (\text{Number of seeds sprouted} / \text{Total number of seeds sprouted}) \times 100$$

Mean germination time (MGT) was determined according to Matthews and Khajeh-Hosseini (2007).

$$\text{Mean germination time} = \sum Gt / \sum t$$

where G is the number of seeds newly germinated at the time of (t), and t is the number of days from sowing.

Seedling vigour index (VI) was determined according to the given formula of Vashisth and Nagarajan (2010).

$$\text{Vigor index (VI)} = \text{germiantion\%} \times \text{mean of seedling length (root + shoot)}$$

Germination index (GI) was calculated according to the formula given by Tao and Zheng (1990).

$$GI = \sum Gt / Dt$$

where Gt is percent germination on each day (t) and Dt represents total number of days for germination.

At the end of the 14 d, after taking seedling fresh weight, samples were then placed in an oven run at 60 °C for 48 h for dry weight of seedling.

Histochemical detection of reactive oxygen species (ROS) in roots of barley seedlings

H₂O₂ was detected using the fluorescent probe 2'-7'- dichlorofluorescein diacetate (DCF-DA) following the method described by Tarpey et al.,(2004). H₂O₂ was detected by incubating roots with 25 μM DCF-DA (prepared in 10 mM Tris-HCl) for 30 min at 37°C. Thereafter, roots were washed in buffer and imaged using a fluorescence microscope at excitation and emission wavelengths of 480 and 530 nm.

Superoxide radicals (O₂^{•-}) were detected in the roots barley seedlings by using 10 μM dihydroethidium (DHE) following the method described by Rodriguez-Serrano et al., (2009). The signal of DHE was captured in the roots as red fluorescence (490 nm excitation; 520 nm emission).

Root viability staining

Roots of each treated plant were collected and stained with fluorescein diacetate (FDA) and propidium iodide (PI). Viable and non-viable cells of primary lateral roots cap weredetectedaccording to the method of Truernit and Haseloff (2008). Root tips were incubated for 20 to 30 min in a solution of 5 μg fluorescein diacetate in 1 mL waterand a solution of 10 μg propidium iodide in 1mL water). Root tips of all samples were removed from solution and examined for viability and non-viability with afluorescence microscope at excitation and emission wavelengths of 488/505 and 530 nm for FDA, and 543 and 585 for PI, respectively.

Anatomical observation of stem of barley seedlings



The samples were collected and stem sections were cut by hand using a sharp blade. The sections were stained with safranin-fast green (counter-dyeing). All sections were observed with fluorescent microscope (Eclipse Ni-U, Nikon, Tokyo, Japan), and images were captured by a DS-Ri1 camera (Nikon, Tokyo, Japan).

Malondialdehyde

In order to measure lipid peroxidation seedlings, malondialdehyde (MDA) concentration was estimated according to the procedure of Dhindsa et al., (1981). MDA concentration was calculated according to Heath and Packer (1968).

Proline

Proline (Pro) concentration in the leaf tissues of barley seedlings was measured spectrophotometrically via reaction with ninhydrin following the method of Bates et al., (1973).

Statistical analysis

All the treatments had four replicates and each Petri dish was treated as one replicate. The statistical analysis was performed using SPSS v17 statistical software (SPSS Inc., Chicago, IL, USA). The data were expressed as means \pm standard error and means were statistically compared by Duncan's multiple-range test (DMRT) at the $p < 0.05$ % level.

RESULTS

Data presented in Table 1 reveal that the application of low level of B increased all germination parameters (G%, VI, GI and MGT) of barley as compared to respective controls. However, high levels of B and NaCl decreased these germination characteristics of barley. In the present study, low levels of B improved these germination traits under NaCl stress. Application of 50 μ M B increased G% by 9.70%, VI by 89.90%, GI by 14.50% and MGT by 39.73% over NaCl treatment.

Table 1 Effect of boron on the percent seed germination (G%), vigor index (VI), germination index (GI) and mean germination time (MGT) of *Hordeum vulgare* under NaCl stress.

Treatments	Parameters			
	G(%)	VI	GI	MGT
Control	72.50 \pm 0.72 ^b	1376.33 \pm 28.86 ^b	72.48 \pm 0.70 ^b	3.17 \pm 0.04 ^b
B1	79.32 \pm 0.64 ^a	1718.34 \pm 30.05 ^a	79.31 \pm 0.64 ^a	4.15 \pm 0.08 ^a
B2	71.25 \pm 0.57 ^b	1176.71 \pm 29.58 ^c	70.59 \pm 0.87 ^b	2.42 \pm 0.09 ^c
NaCl stress	60.23 \pm 0.50 ^d	0429.41 \pm 19.38 ^f	58.57 \pm 0.80 ^e	1.46 \pm 0.07 ^f
B1+NaCl	66.07 \pm 0.44 ^c	0815.47 \pm 14.39 ^d	67.06 \pm 0.82 ^c	2.04 \pm 0.05 ^d
B2+NaCl	68.02 \pm 0.84 ^c	0670.51 \pm 08.16 ^e	64.41 \pm 0.71 ^d	1.77 \pm 0.04 ^e

Means of the each parameters followed similar letter within the column are not significantly different at the 0.05 level of probability by Duncan's Multiple-Range Test.

Under non-stress condition, application of B (50 μ M) significantly increased length of seedlings and other growth parameters of barley (Fig. 1 and Table 2). Under NaCl stress condition, barley seedlings had reduced all growth parameters, such as RL, SL, RFW, SFW, RDW and SDW. However, application low dose of B significantly improved RL, SL, RFW, SFW, RDW and SDW by 76.95, 49.58, 183.33, 101.88, 100 and 50%, respectively over the NaCl treatment.

Figure 2A and B shows the *in situ* production of ROS (H_2O_2 and $O_2^{\cdot-}$ detected using DCF-DA and DHE respectively) in root of barley seedlings under stress and non-stress conditions. Under non-stress condition, low



levels of B gave relatively weak signal of green DCF fluorescence and DHE red fluorescence compared with high signal intensity of control. Also, NaCl treated root of barley seedlings exhibited a strong signal as compared to all treated roots. Also, Figure 2 C reveal that the apical cells of elongation zone of root treated with NaCl and high concentration of B were more severely damaged as compared with the mature zone (as produced strong green signal by FDA fluorescence probe; viable cells). Root treated with 50 μM B exhibited weak red signal of PI fluorescence (non-viable cells) as compared to roots treated with NaCl and high levels of B. Under NaCl stress. The viability loss in 50 μM B treated roots was detected lower as compared to viability loss in NaCl and 100 μM B treated of roots of seedlings.

Table 2. Effect of boron on root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW) and shoot dry weight (SDW) of *Hordeum vulgare* under NaCl stress.

Treatments	Parameters					
	RL (cm)	SL (CM)	RFW (g)	SFW (g)	RDW (g)	SDW (g)
Control	6.25±0.26 ^b	15.70±0.35 ^b	0.032±0.002 ^b	0.132±0.002	0.009 ^a	0.0163±0.00012 ^b
B1	7.00±0.07 ^a	18.23±0.72 ^a	0.039±0.001 ^a	0.161±0.005	0.008 ^a	0.020±0.00058 ^a
B2	5.68±0.35 ^{bc}	15.10±0.55 ^b	0.035±0.002 ^{ab}	0.122±0.002	0.006 ^b	0.013±0.00058 ^c
NaCl stress	3.08±0.14 ^e	08.43±0.57 ^e	0.012±0.001 ^c	0.053±0.004	0.003 ^d	0.008±0.00058 ^e
B1+NaCl	5.45±0.26 ^c	12.61±0.47 ^c	0.034±0.0003 ^b	0.107±0.003	0.006 ^b	0.012±0.00033 ^{cd}
B2+NaCl	4.12±0.14 ^d	10.73±0.533 ^d	0.030±0.002 ^b	0.092±0.002	0.004 ^c	0.010±0.00033 ^d

Means of the each parameters followed similar letter within the column are not significantly different at the 0.05 level of probability by Duncan's Multiple-Range Test.

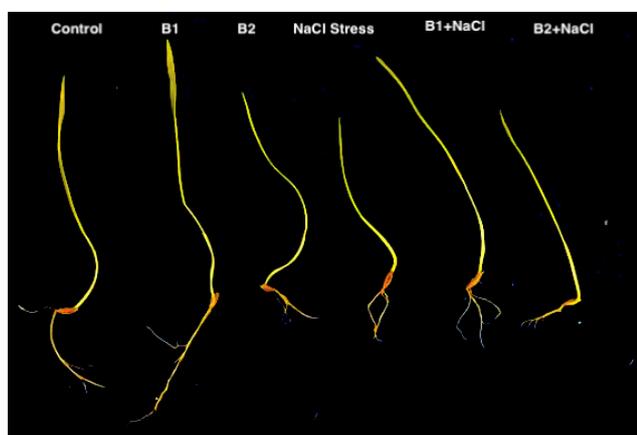


Figure 1. Effect of boron on the length of seedlings of *Hordeum vulgare* under NaCl stress.

Under non-stress condition, accumulation of MDA decreased in seedlings of barley treated with 50 μM B over the control (Fig. 3). However, NaCl and 100 μM B treated barley seedlings exhibited a significant increase in MDA content compared to control. Under NaCl stress, low dose of B significantly decreased accumulation of MDA in barley seedlings. Application B (50 μM) decreased MDA content by 40.68% over the NaCl treatment.

Figure 4 reveals that a clear change in the shape and size of vascular tissue and endodermis was observed in the stem section of both control and treated barley seedlings. NaCl treatment in the presence of low and high levels of B did not cause major changes in the organization of root tissue of barley seedlings after 2 week under NaCl stress.

Under non-stress condition, accumulation of photosynthetic pigments increased in the leaves of barley seedlings with 50 μM B over the control (Fig. 5 A, B and C). Moreover, application of NaCl and 100 μM B



significantly decreased the synthesis of pigments (Chl a , b and total Chl. Application of low levels of B was found to be effective in improving the accumulation photosynthetic pigments under NaCl stress. Treatment B (50 μ M) increased Chl a by 107.52%, b by 88.05% and total Chl 109.75% over the NaCl treatment.

Figure 6 reveals that applications of B increased Pro accumulation under both NaCl stress and non-stress conditions. Also, application NaCl increased Pro content in barley seedlings. However, application of low levels of B (50 μ M) significantly improved Pro content by 94.34% over the NaCl treatment.

DISCUSSION

Application of low concentration of boron (50 μ M) to barley seedlings substrate generally improved germination, growth, physiological and biochemical characteristics (Tables 1 and 2, and Figs. 1, 5 and 6); however, high levels of B and NaCl were toxic to barley.

It is fact that seed germination is regulated by a number of mechanisms, and good germination of seed insures the production of a new healthy plant. In the present experiment, application of NaCl caused inhibition of barley seed germination (Table 1). This may be overcome by inclusion of low concentration of B that induced seed potential by increasing seed germination parameters (G%, VI, GI and MGT) under both NaCl stress and non-stress conditions. However, high dose of B and NaCl caused toxicity to seed germination, it may due to the over production of ROS (Fig. 2 A and B). Under NaCl stress, low concentration of B (50 μ M) improved all germination traits; it may be due to the role of B in stimulation of seed germination by breaking seed dormancy and activating the activity of *alpha*-amylase in embryo and endosperm and also by increasing RNA levels (Cresswell and Nelson, 1973).

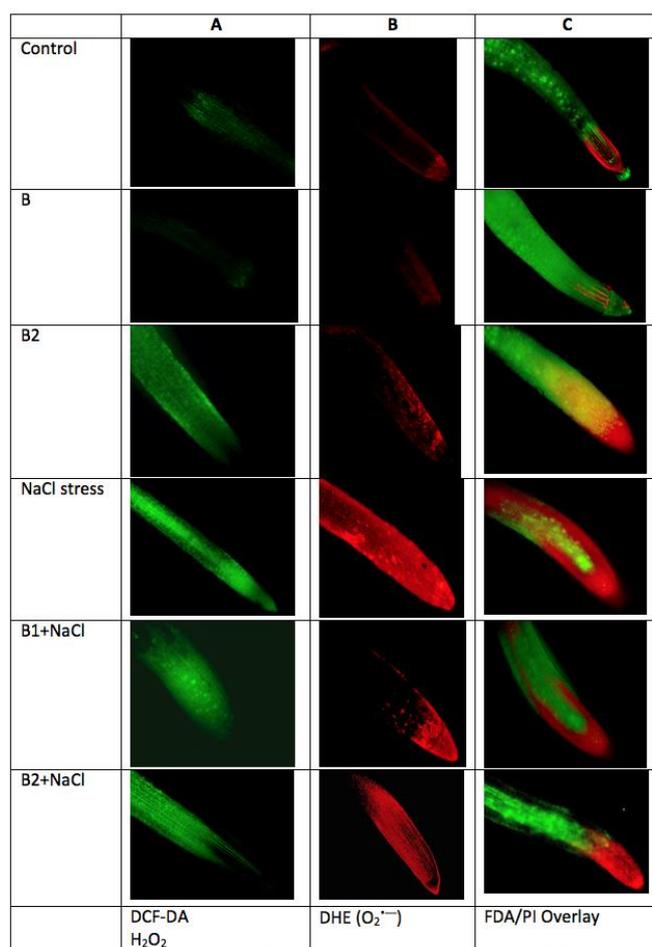


Figure 2. *In situ* visualization of ROS formation in primary roots using the fluorescent probe DCF-DA and DHE for (A) H₂O₂ and (B) (O₂^{•-}) respectively under salinity stress. FDA and PI staining of viable and non-viable primary root cap cells respectively; (C) overlay projection image of root stained with FDA (green) and PI (red).

Low levels of B to barley seedlings enhanced plant growth under NaCl stress and non-stress conditions (Table 2 and Fig. 1). This beneficial effect may be explained by its physiological roles; it helps in cell wall formation and



plant tissue growth, because it is an important component of pectin rhamnogalacturonan II (a low-molecular-mass pectic polysaccharide) (Matoh 1997; O'Neill et al., 2004) and also play a key role in the synthesis of macromolecules (nucleic acid, proteins, carbohydrates) and hormone (Goldbach et al., 2001; Jehangir et al., 2017). A high concentration of B and NaCl decreased growth parameters; it may be due to their toxic effects on root cell division, photosynthetic pigments synthesis (Liu and Yang 2000; Herrera-Rodríguez et al., 2010; Khan et al., 2007; Siddiqui et al., 2009; 2013), and also they caused the formation of ROS (Fig. 2 A and B), resulting death of cells (Fig. 2 C).

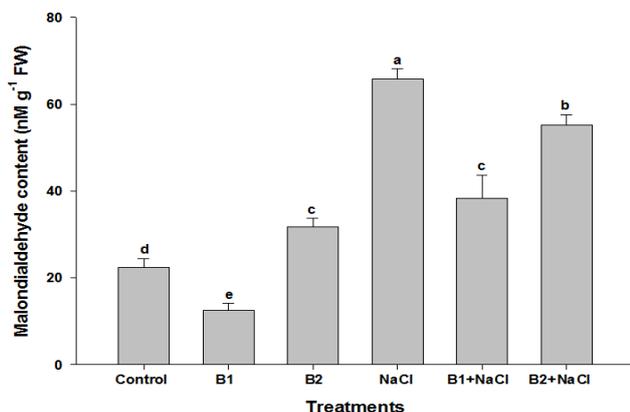


Figure 3. Effect of boron on the content of malondialdehyde in *Hordeum vulgare* under NaCl stress. Bars followed by the same letter do not differ statistically at $p < 0.05$ (Duncan Multiple Range Test). Average of four determinations are presented with bars indicating S.E. Control.

The oxidative damage due to the toxicity of NaCl and high dose of B was measured by detecting of ROS (H_2O_2 and $O_2^{\cdot-}$) in roots and estimating the accumulation of MDA in seedlings of barley (Figs. 2 A-C and 3). The increased values found for accumulation of MDA, and formation of ROS under both levels of NaCl and B (100 μ M) were indicative of NaCl and B-activated cellular dysfunction through the production of lipid peroxidation that caused the death of cells (Fig. 2 C). An increase in MDA and H_2O_2 and $O_2^{\cdot-}$ due to B and NaCl was also observed by Siddiqui et al., (2009; 2013). However, low dose of B (50 μ M) suppressed the production of ROS in barley seedlings; it may be due to the accumulation of Pro (Fig. 6) and also B improves the activity of antioxidant enzymes that scavenge ROS under stress (Siddiqui et al., 2013). In the present study, alterations in tissue organization were investigated for stem of barley seedlings under NaCl stress and high levels of B (Fig. 4). The result of this study complements other findings (Bastias et al., 2015; Ma et al., 2015) and confirmed the B-induced tolerance to NaCl.

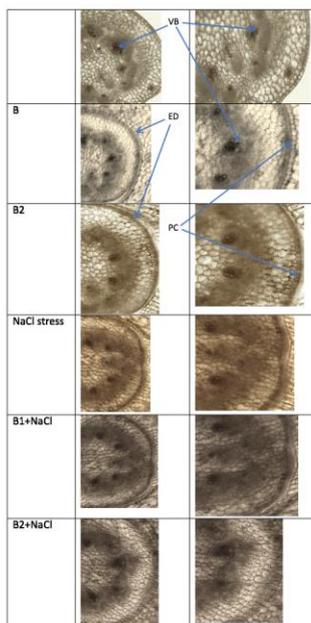


Figure 4. Effect of boron on the stem structure of seedlings of *Hordeum vulgare* under NaCl stress. VB: vascular bundle, ED: endodermis, PC: pericycle

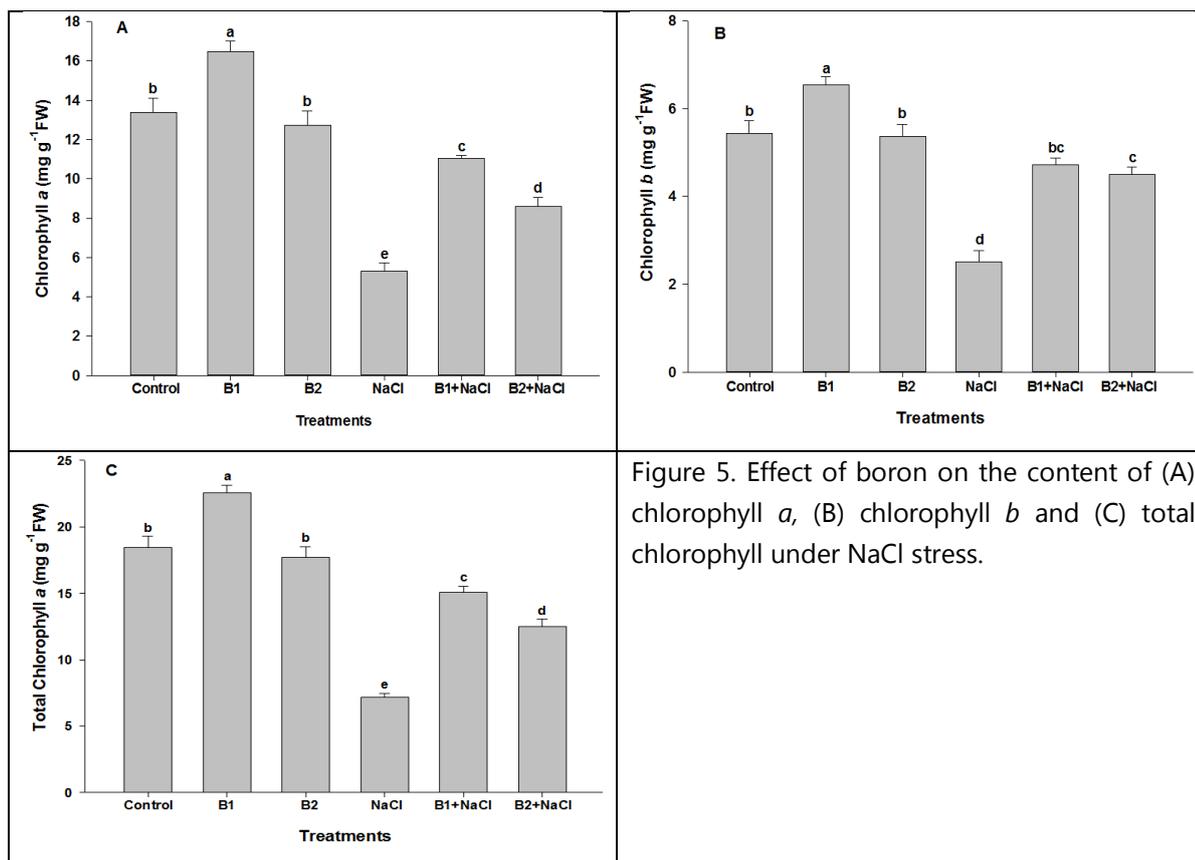


Figure 5. Effect of boron on the content of (A) chlorophyll *a*, (B) chlorophyll *b* and (C) total chlorophyll under NaCl stress.

Photosynthetic pigments are important components in chloroplast and regulate the key process photosynthesis in plant. In the present study, the synthesis of photosynthetic pigments (Chl*a* and *b* and total Chl) was severely affected by the NaCl stress and the higher dose of B (100 μ M) (Fig. 5 A-C). This may be due to lipid peroxidation (Fig. 3) that could be the reason for oxidative damage of chloroplast and damage of reaction centers (Kyle, 1987). Siddiqui et al., (2013) reported that a high concentration of B caused inhibition of pigments synthesis. Also, high levels of B cause photooxidation damage to macromolecules (Cervilla et al., 2007). However, a low concentration of B (50 μ M) improved tolerance of barley seedlings to NaCl stress by increasing pigments synthesis (Fig. 5 A-C). An increase in the content of Chl*a* and *b* and total Chl may be due to the involvement of B in maintenance of cell integrity and improvement of antioxidant system that could protect chloroplast from oxidative damage (Xuan et al., 2001).

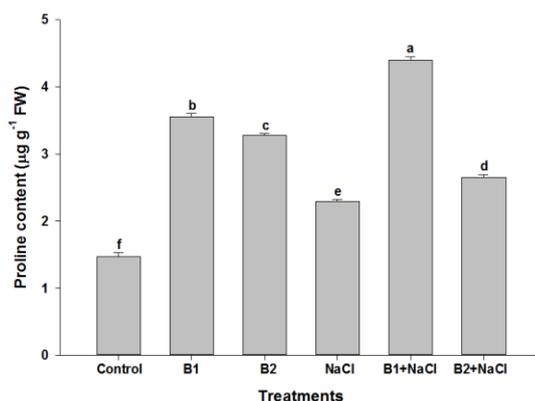


Figure 6. Effect of boron on the content of proline in *Hordeum vulgare* under NaCl stress. Bars followed by the same letter do not differ statistically at $p < 0.05$ (Duncan Multiple Range Test). Average of four determinations are presented with bars indicating S.E. Control.

Compatible solutes are very important in plant to maintain osmotic adjustment during abiotic stress by lowering and balancing osmotic potential within the cells. In our study, Pro levels increased relative to the control when plant supplied a higher dose of B and NaCl (Fig. 6). At low concentration of B, Pro accumulation increased



further under NaCl stress. Increased Pro in barley seedlings at low levels of B may be one of the reasons for the tolerance of barley seedlings to salinity because Pro acts like an antioxidant and source of N and C and also regulates osmotic adjustment associated genes (Matysik et al., 2002; Iyer and Caplan 1998).

CONCLUSION

In conclusion, low levels of B significantly increased seed germination and growth of barley seedlings and NaCl and a higher dose of B caused inhibition of germination and growth characteristics and alteration in anatomical structure of barley seedlings. Results of this study reveal that pigments synthesis decreased and formation of ROS, lipid peroxidation and non-viable cells increased on the treatment with a high concentration of B and NaCl. However, low levels of B (50 μ M) significantly inhibited the inhibitory effects of NaCl by reducing lipid peroxidation and the production of non-viable cells in roots through accumulation Pro. Also, a low concentration of B improves photosynthetic pigment synthesis under both stress and non-stress conditions.

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