INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–1242/2019/21–6–1271–1278
DOI: 10.17957/IJAB/15.1021
http://www.fspublishers.org

Full Length Article

Assessment of Gas Exchange Attributes, Chlorophyll Contents, Ionic Composition and Antioxidant Enzymes of Bread Wheat Genotypes in Boron Toxic, Saline and Boron Toxic-Saline Soils

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Abstract

Combined salinity and toxic levels of B are usually found in the soils and ground water of arid and semi-arid regions. A pot study was conducted to evaluate the effect of combined stresses of B toxicity and salinity on the growth, yield, physiological and biochemical processes of wheat. The study comprised of twelve treatments including four levels of B (control, 2.5, 5 and 7.5 mg kg^{-1}) and three levels of salinity (control, 100 and 200 m*M* NaCl). The results showed that at lower level of B *i.e*., 2.5 mg kg^{-1}, the growth, yield and physiological attributes of wheat were improved at both levels of salinity. While the higher B levels (5 and 7.5 mg kg^{-1}) and salinity together reduced wheat growth, photosynthetic and transpiration rates, stomatal conductance and yield. However, this decrease was higher in sensitive wheat genotype than tolerant one. The activity of antioxidant enzymes increased with increasing salinity and B stresses either alone or in combination. An antagonistic salinity-B interaction was observed as the reduction in growth and yield parameters in the presence of combined salinity and toxic B levels was less than the sum of reduction caused by individual salinity and toxic B.

Keywords: Photosynthetic rate; Transpiration rate; Stomatal conductance; SPAD-values; Cationic/Anionic composition; Superoxide dismutase; Catalase; Salt-affected B-spiked soils
Abbreviations: CAT-Catalase; PR- Photosynthetic rate; ROS-Reactive oxygen species; SC-Stomatal conductance; SOD-Superoxide dismutase; TCC-Total chlorophyll content; TR-Transpiration rate

Introduction

Soil salinity is one of the major abiotic stress factor limiting agricultural productivity around the globe (Flowers, 2004). Out of the total (79.61 mha) geographical area of Pakistan, 31.23 mha is cultivated, of which 11.5 mha is salt-affected (FAO, 2005). About 56% of the salt-affected soils of Pakistan and 84% those of Punjab are saline-sodic in nature. In Punjab province about 75–80% of the pumped ground water is unfit for irrigation owing to high residual sodium carbonate (RSC), electrical conductivity (EC) and/or sodium adsorption ratio (SAR) (Ghafoor *et al*., 2001). A range of dissolved salts are present in the soil solution of salt affected soils including NaCl, $CaSO_4$, Na_2SO_4, $MgCl_2$, Na_2SO_4, KCl and Na_2CO_3 but NaCl is the most soluble and prevalent salt (Rengasamy, 2002; Munns and Tester, 2008). Salinity affects the crop growth in two phases. In the first phase, water availability to plants is decreased because of the reduced osmotic potential of the soil solution. In the second phase, toxicity of ions such as Na^+, Cl^- or B occurs due to the accumulation of these ions in excess concentrations (Yamaguchi and Blumwald, 2005). Salinity also affects physiological processes in plants such as photosynthesis by decreasing stomatal conductance (SC) and transpiration, and disturbing the biosynthesis of photosynthetic pigments (Sairam *et al*., 2005).

Boron (B) is an essential micronutrient which affects major metabolic events and cellular functions in plants (El-Hamdaoui *et al*., 2003). It is required by actively growing regions of the plants where cells divide and differentiate rapidly thus plays a critical role in growth and development. However, role of B in physiological processes including photosynthesis is unclear and sometimes it is contradictory.

To cite this paper: Naz, T., J. Akhtar, M.M. Iqbal, M. Anwar-ul-Haq, G. Murtaza, N.K. Niazi, Atique-ur-Rehman, O. Farooq, M. Ali and B. Dell, 2019. Assessment of gas exchange attributes, chlorophyll contents, ionic composition and antioxidant enzymes of bread wheat genotypes in boron toxic, saline and boron toxic-saline soils. *Intl. J. Agric. Biol*., 21: 1271–1278

There have been no reports on the direct effect of B on the photosynthesis of plant (Dell and Huang, 1997), but some investigators stated indirect association of B with photosynthesis in crop plants such as soybean (Liu *et al*., 2005). The B excess (Chen *et al.*, 2012) and deficiency (Kastori *et al*., 1995; Sheng *et al*., 2009), the both condition decrease the rate of photosynthesis. B excess reduce CO_2 assimilation that appears to be correlated to a combination of different reasons *viz*., oxidative load, reduction in activities of photosynthetic enzymes and impaired electron transport rate (Han *et al*., 2009).

Boron causes oxidative stress in plants when it is deficient or present in excess, which is responsible for the over production of reactive oxygen species (ROS) (Han *et al*., 2009). These ROSs (*viz*., O_2^-) and the radicals derived from ROS (*viz*., H_2O_2, OH^-) are strongly toxic to plants, may cause lipid peroxidation and damage to cellular membranes, protein denaturation and genotoxic effects *i.e*., DNA mutation (Zhang *et al*., 2011). Plants have evolved a well-equipped antioxidant defense mechanism consisting of enzymatic antioxidants and no-enzymatic antioxidants which normally neutralized ROS molecules under steady state condition (Foyer and Noctor, 2005) and consequently reduce cellular damage. In plants, superoxide dismutase (SOD) and catalase (CAT) are two important protective antioxidant enzymes against ROS. However, there are limited reports, which are a bit conflicting, related to antioxidant response of plants to B-toxicity (Molassiotis *et al*., 2006) and B-deficiency (Han *et al*., 2008).

Salinity and B toxicity often coincides with each other, especially in arid or semi-arid region (Grieve and Poss, 2000). So far, no consensus is found in literature regarding the interactive effect of salinity and B deficiency or toxicity on plant growth and development, and results regarding the combination of these stresses are inconsistent. Some studies described additive effects of salinity and B stresses (Wimmer *et al*., 2003; Masood *et al*., 2012) while other reports suggested independency of the interaction (Grattan *et al*., 1996; Edelstein *et al*., 2005). Moreover, antagonistic effects were also reported (Bastias *et al*., 2004; Yermiyahu *et al*., 2008).

In general, it is expected that crop species/genotypes having better salt tolerance could absorb and accumulate less B under B-toxic conditions (Nable *et al*., 1997). Variation in salinity and B accumulation amongst wheat varieties has been previously observed (Wimmer and Goldbach, 2012). Thus, successful wheat crop production with better grain quality in saline and toxic or deficient B conditions demands the selection of suitable genotype having better salt and B tolerance.

To-date a very limited research work is available regarding the growth, physiological and biochemical responses of salt tolerant and sensitive wheat genotypes exposed simultaneously to salinity and B toxic or deficient levels. Therefore, the present pot study was conducted to evaluate the growth, physiological and antioxidative enzymes responses of two bread wheat genotypes (differing in tolerance to salinity) exposed to exogenously applied soil salinity, varying B rates and their combined application.

Materials and Methods

Growth Conditions and Treatments

An experiment was conducted in pots in the wire house having a glass covered roof (sides open having only iron wire screen and no control over temperature and humidity) in the University of Agriculture Faisalabad Pakistan. In the wire house, the recorded average temperature was 14.5°C and relative humidity was 66.5% during winter seasoned pot study having day and night temperature ranging from 26.3°C maximum to 2.7°C minimum. The soil used in the experiment was sandy loam in texture (59.8% sand, 24.8% silt and 16.3% clay), with a pH of 7.7, 0.96 dS m^{-1} EC (saturation extract), 2.96 (mmol $L^{-1})^{1/2}$ SAR, 0.72% organic matter and 0.39 mg B kg^{-1} of soil (hot water soluble). This pot study comprised of twelve treatments as: T_1 = Control, T_2 = B at 2.5 mg kg^{-1}, T_3 = B at 5 mg kg^{-1}, T_4 = B at 7.5 mg kg^{-1}, T_5 = EC 10 dS m^{-1}, T_6 = EC 10 dS m^{-1} + B at 2.5 mg kg^{-1}, T_7 = EC 10 dS m^{-1} + B at 5 mg kg^{-1}, T_8 = EC 10 dS m^{-1} + B at 7.5 mg kg^{-1}, T_9 = EC 20 dS m^{-1}, T_{10} = EC 20 dS m^{-1} + B at 2.5 mg kg^{-1}, T_{11} = EC 20 dS m^{-1} + B at 5 mg kg^{-1}, T_{12} = EC 20 dS m^{-1} + B at 7.5 mg kg^{-1}. The treated pots were arranged in completely randomized design (CRD), with three replications each.

The soil was artificially made salt-affected (*i.e.*, ECe = 10 and 20 dS m^{-1}) using NaCl salt by adding 20.15 and 42.3 NaCl g per pot respectively. After this step, the designed levels of B using H_3BO_3 were added to make specified salt-affected B-spiked soil (Eraslan *et al*., 2007). Each pot was filled with 12 kg soil and no leaching provision was maintained. The seeds of two bread wheat genotypes (*i.e*., SARC-I-salt tolerant and MH-97-salt sensitive) (Saqib, 2002) were taken from gene bank of the Saline Agriculture Research Centre UAF and Ayub Agricultural Research Institute Faisalabad. In present study, ten seeds of both wheat genotypes were planted per respective pot. After one week of germination, six plants per pot were retained and uprooted plants were crushed and mixed into their respective pots. Wheat crop was fertilized at 120 – 90 – 60 NPK kg ha^{-1} as urea, di-ammonium phosphate and potassium sulfate, respectively. All the P, K and half of the N, were applied at sowing while rest of N was applied in two equal splits; 30 and 45 days after sowing. Distilled water was used to irrigate the crop as and when required. At maturity, wheat crop was harvested and plant height, straw and grain yields were recorded. Wheat leaf samples were collected for further chemical analyses.

Measurement of Physiological Responses

After 60 days of sowing, measurements on physiological

processes like net photosynthetic rate (PR), transpiration rate (TR) and stomatal conductance (SC) were made (from 10:00 a.m. to 1:00 p.m.) on fully extended leaves of randomly selected three wheat plants from each pot using portable narrow chambered infrared gas analyzer (IRGA, LCA-4, Analytical Development Company, Hoddesdon, England) following Iqbal *et al.* (2015).

Wheat flag leaf total chlorophyll content (TCC) index in terms of Special Products Analysis Division (a SPAD, division of Minolta) value was determined from leaf tip to leaf base *via* a hand-held SPAD-502 meter (Minolta, Osaka, Japan) and then averaged following Saqib *et al.* (2012).

Enzyme Extraction and Assays

The fresh leaf samples (0.1 g) were ground using a mortar pestle in 5 mL of 50 m*M* cooled phosphate buffer (pH 7.8) placed in an ice bath. The homogenate was centrifuged at 15000 g for 20 min at 4°C. The supernatant was used for the determination of antioxidant enzymes.

The SOD was assayed by the nitroblue tetrazolium (NBT) method as described by Gong *et al.* (2005). The reaction mixture (3 mL) contained 50 m*M* K-phosphate buffer, pH 7.3, 13 m*M* methionine, 75 m*M* NBT, 0.1 m*M* EDTA, 4 m*M* riboflavin and enzyme extract (0.2 mL). Riboflavin was added lastly, and the glass test tubes were shaken and placed under fluorescent lamps (60 mmol m^{-2} s^{-1}). The reaction was allowed for 5 min and then absorbance was measured at 560 nm. Blanks and controls were run in the same manner but without illumination and enzyme extract, respectively. One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under the assay conditions.

The CAT activity was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2 (Cakmak *et al*., 1993). The reaction mixture (3 mL) contained 50 m*M* phosphate buffer (pH 7.0), 15 m*M* H_2O_2 and 50 μL of crude enzyme extract at 25°C. The activity was calculated from the extinction coefficient (40 mM^{-1} cm^{-1}) for H_2O_2.

Determination of Ionic Parameters

At harvest all the four wheat plants per respective pot were cut at the bottom of stem, leaves were separated and dried for analysis. To assess the B, Na^+, K^+ and Cl^- concentration, the leaf samples were oven dried at 70°C in an air forced oven for 48–72 h and subsequently ground with plant grinding Willey mill.

For dry ashing, 0.50 g of the ground leaf samples was taken in porcelain crucibles and were placed in a typical muffle furnace, and the temperature was increased from 400 to 550ºC and the ashing process was continued for 5 h after attaining 550ºC. The ashed samples were wetted with 8–10 drops of deionized water and subsequently 10 mL 0.36 *N* H_2SO_4 was added in the crucible. After 50 min, samples were stirred and filtered through Whatman No. 1 filter paper and diluted with distilled water to make 50 mL total volume and stored in plastic bottles (Chapman and Pratt, 1961). This filtrate was used for the determination of Na^+, K^+, Cl^- and B contents.

The concentration of B in the leaf extract was measured using azomethine-H as described by Bingham (1982). This is a colorimetric method in which azomethine-H is used as a color developing reagent and the intensity of color developed is recorded with UV-vis-spectrophotometer at 420 nm wave length. The leaf extract was diluted by adding distilled water and Na^+ and K^+ were determined with Sherwood 410 Flame Photometer with the help of prepared standard solutions using reagent grade NaCl and KCl. The Cl^- in leaf samples was determined titrimetrically (Estefan *et al.*, 2013).

Results

Growth and Yield Responses

Growth traits such as plant height (Fig. 1a), straw dry matter (Fig. 1b) and grain yield (Fig. 1c) of salinity and B stressed wheat were significantly ($p \leq 0.05$) affected depending on B level, salinity and wheat genotype. Soil salinity resulted in more reduction of plant growth and was found as the dominant stress factor in this study. The presence of salinity at both levels (*i.e.*, 10 and 20 dS m^{-1}) significantly reduced plant growth as compared to control. The grain yield of wheat was increased with 2.5 mg kg^{-1} B application in non-saline soil and at soil EC level of 10 dS m^{-1}. However, at 10 and 20 dS m^{-1} EC levels, there was no significant difference in plant height and straw dry matter with the application of 2.5 mg kg^{-1} B. With increasing levels of B (5 and 7.5 mg kg^{-1}), the plant growth was decreased gradually. Reduction in growth due to the combined salinity and B toxicity was less than the sum of reduction caused by the individual stress factors. The decrease in growth was higher in sensitive genotype than tolerant one in response to combined stresses of B and salinity.

Physiological Responses

The physiological parameters such as TCC (Fig. 2a), PR (Fig. 2b), TR (Fig. 2c) and SC (Fig. 2d) were significantly affected by salinity, B and the combined salinity and B stress. Data showed that with 2.5 mg B kg^{-1} soil, the TCC, TR and SC was not severely affected under both normal and salt-affected soils. However, the PR in SARC-I was significantly increased at this treatment under both non-saline and saline conditions. The application of B at higher rates *i.e.*, 5 and 7.5 mg kg^{-1} soil, reduced TCC, PR, TR and SC in both genotypes in non-saline and saline condition. The presence of salinity alone as well as with higher B levels *i.e.*, 5 and 7.5 mg kg^{-1} further aggravated

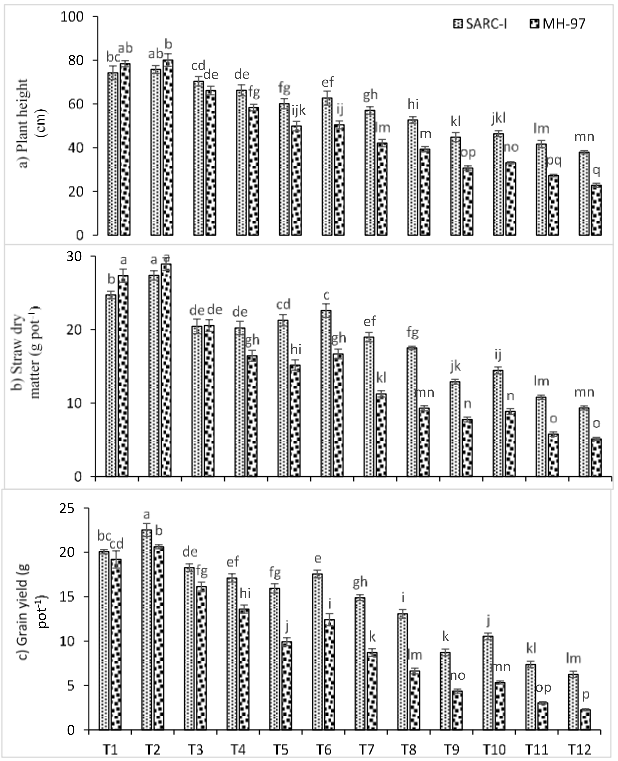

Fig. 1: Growth and yield responses (a = plant height, b = straw dry matter, and c = grain yield) of sensitive and tolerant bread wheat genotypes in B toxic, saline and B toxic-saline soils (Means ± SE, n = 3)

[LSD for plant height: V = 1.5*, T = 3.68*, V × T = 5.20*; LSD for straw dry matter: V = 0.52*, T = 1.27*, V × T = 1.81*; LSD for grain yield: V = 0.40*, T = 0.95*, V × T = 1.36*]

[Whereas V = Genotype, T = Treatment, NS = Non-significant ($P > 0.05$), * = Significant ($P \leq 0.05$), ** = Highly significant ($P \leq 0.01$)]

Treatments: T_1 = Control, T_2 = B at 2.5 mg kg^{-1}, T_3 = B at 5 mg kg^{-1}, T_4 = B at 7.5 mg kg^{-1}, T_5 = EC 10 dS m^{-1}, T_6 = EC 10 dS m^{-1} + B at 2.5 mg kg^{-1}, T_7 = EC 10 dS m^{-1} + B at 5 mg kg^{-1}, T_8 = EC 10 dS m^{-1} + B at 7.5 mg kg^{-1}, T_9 = EC 20 dS m^{-1}, T_{10} = EC 20 dS m^{-1} + B at 2.5 mg kg^{-1}, T_{11} = EC 20 dS m^{-1} + B at 5 mg kg^{-1}, T_{12} = EC 20 dS m^{-1} + B at 7.5 mg kg^{-1}

the reduction in physiological parameters. Among tested wheat genotypes, SARC-I showed significantly higher TCC, PR, TR and SC than the MH-97 under salinity and B stress conditions.

Concentration of B, Na^+, K^+ and Cl^-

In present pot study, the concentration of B (Fig. 3a) in wheat leaves was significantly affected by B, salinity and their interaction. The leaf B concentration was increased with increasing B in both normal and salt-affected soils. The increasing soil salinity decreased the leaf B concentration than that found in normal B-toxic soil. The leaf B concentration was higher in sensitive genotype while the tolerant genotype maintained low level of leaf B.

The leaf Na^+ (Fig. 3b) concentration increased while K^+ concentration (Fig. 3c) decreased with increasing soil salinity. The increasing B had no influence on leaf Na^+

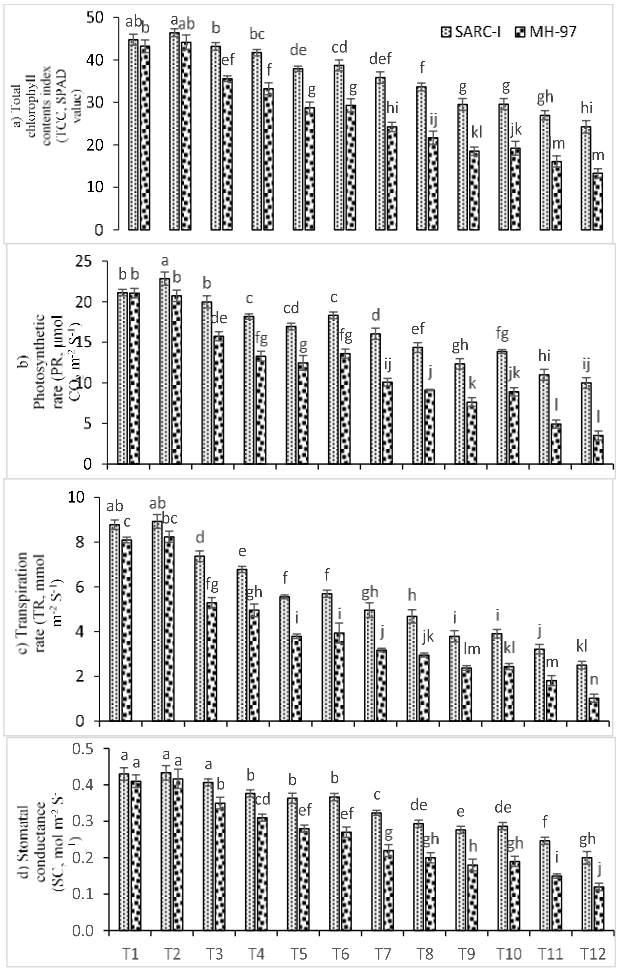

Fig. 2: Physiological responses (a = total chlorophyll contents index, b = photosynthetic rate, c = transpiration rate, and d = stomatal conductance) of sensitive and tolerant bread wheat genotypes in B toxic, saline and B toxic-saline soils (Means ± SE, n = 3)

[LSD for total chlorophyll contents index: V = 0.91*, T = 2.20*, V × T = 3.11*; LSD for photosynthetic rate: V = 0.42*, T = 1.03*, V × T = 1.46*; LSD for transpiration rate: V = 0.16*, T = 0.40*, V × T = 0.57*; LSD for stomatal conductance: V = 0.008*, T = 0.02*, V × T = 0.03*]

[Whereas V = Genotype, T = Treatment, NS = Non-significant ($P > 0.05$), * = Significant ($P \leq 0.05$), ** = Highly significant ($P \leq 0.01$)]

Treatments: T_1 = Control, T_2 = B at 2.5 mg kg^{-1}, T_3 = B at 5 mg kg^{-1}, T_4 = B at 7.5 mg kg^{-1}, T_5 = EC 10 dS m^{-1}, T_6 = EC 10 dS m^{-1} + B at 2.5 mg kg^{-1}, T_7 = EC 10 dS m^{-1} + B at 5 mg kg^{-1}, T_8 = EC 10 dS m^{-1} + B at 7.5 mg kg^{-1}, T_9 = EC 20 dS m^{-1}, T_{10} = EC 20 dS m^{-1} + B at 2.5 mg kg^{-1}, T_{11} = EC 20 dS m^{-1} + B at 5 mg kg^{-1}, T_{12} = EC 20 dS m^{-1} + B at 7.5 mg kg^{-1}

while leaf K^+ concentration increased significantly with increasing B in both saline and non-saline conditions. The tolerant genotype maintained higher leaf K^+ and lower leaf Na^+ concentration than the sensitive genotype.

The concentration of Cl^- (Fig. 3d) in wheat leaves was also significantly affected salinity, B and their interaction. The increasing soil salinity resulted in increased leaf Cl^-

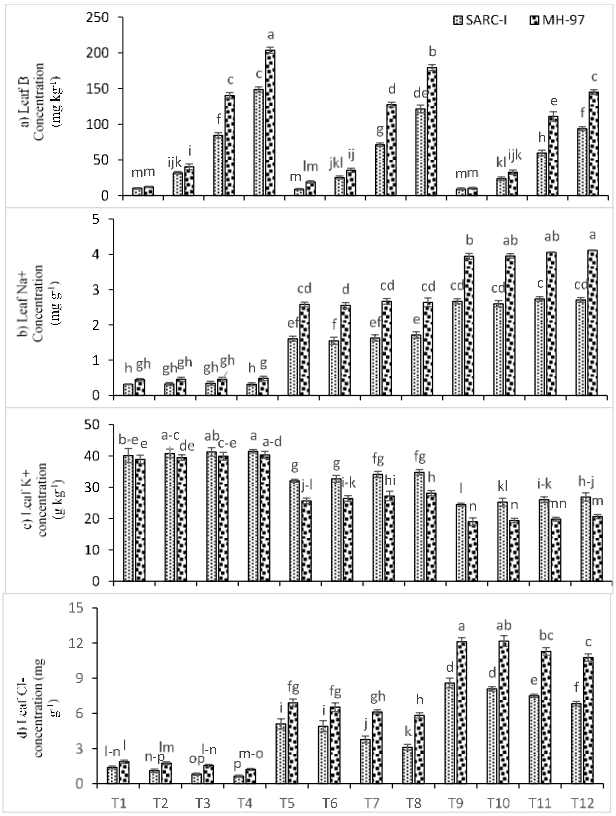

Fig. 3: Ionic composition (a = leaf B concentration, b = leaf Na^+ concentration, c = leaf K^+ concentration, and d = leaf Cl^- concentration) of sensitive and tolerant bread wheat genotypes in B toxic, saline and B toxic-saline soils (Means ± SE, n = 3)

[LSD for leaf B concentration: V = 3.11*, T = 7.58*, V × T = 10.73*; LSD for leaf Na^+ concentration: V = 0.05*, T = 0.11*, V × T = 0.16*; LSD for leaf K^+ concentration: V = 0.36*, T = 0.89*, V × T = 1.26*; LSD for leaf Cl^- concentration: V = 0.16*, T = 0.40*, V × T = 0.56*]

[Whereas V = Genotype, T = Treatment, NS = Non-significant ($P > 0.05$), * = Significant ($P \leq 0.05$), ** = Highly significant ($P \leq 0.01$)]

Treatments: T_1 = Control, T_2 = B at 2.5 mg kg^{-1}, T_3 = B at 5 mg kg^{-1}, T_4 = B at 7.5 mg kg^{-1}, T_5 = EC 10 dS m^{-1}, T_6 = EC 10 dS m^{-1} + B at 2.5 mg kg^{-1}, T_7 = EC 10 dS m^{-1} + B at 5 mg kg^{-1}, T_8 = EC 10 dS m^{-1} + B at 7.5 mg kg^{-1}, T_9 = EC 20 dS m^{-1}, T_{10} = EC 20 dS m^{-1} + B at 2.5 mg kg^{-1}, T_{11} = EC 20 dS m^{-1} + B at 5 mg kg^{-1}, T_{12} = EC 20 dS m^{-1} + B at 7.5 mg kg^{-1}

concentration. The concentration of Cl^- in wheat leaf decreased significantly with increasing B in normal and salt-affected soils. The concentration of Cl^- was higher in sensitive genotype than the tolerant one.

Activity of SOD and CAT

Soil salinity, B and their interactive effects had a significant ($P \leq 0.05$) influence on the activity of SOD (Fig. 4a) and CAT (Fig. 4b). The activity of SOD and CAT increased with increasing B and soil salinity. The increase in activity of enzymes was higher with increasing salinity than with increasing B. the tolerant genotype had higher activity of both enzymes under soil salinity and B toxicity than the sensitive genotype.

Discussion

Many previous research works have indicated variable opinions about the effects of B toxicity and salinity on plants growth. Ismail (2003) described that severity of B toxicity is reduced by salinity because of reduced accumulation of B in shoot and stem of sorghum. In disparity, Alpaslan and Gunes (2001) reported more reduction in the growth of cucumber and tomato by combined B toxicity and salinity than by the individual stresses. Therefore, present study was conducted to determine the growth, physiological, ionic and antioxidative enzymes responses of two wheat genotypes exposed to NaCl salinity, B toxicity and their combined stresses. In present study, straw dry matter and grain yield of wheat were determined as indicators of wheat growth against applied soil salinity, B and their combination. The growth parameters and yield were increased at lower level of B (*i.e.*, 2.5 mg kg^{-1}) in both normal and salt-affected soils. This might be due to the reason that moderate soil salinity interact positively with the plant nutrients such as B and could enhance the metabolism resulting in normal plant growth (Iqbal *et al*., 2017). It has also been previously reported that that dry matter production and seed yield in rapeseed increased with B level upto 4.5 mg kg^{-1} (Hossain *et al*., 2015).

The decrease in growth and yield of wheat in current study with increasing B levels was due to the interruption of different metabolic processes like photosynthesis, transpiration and stomatal conductance, ultimately resulting in poor growth leading to low biomass and yield. In the present study, decrease in photosynthesis under salinity and B stresses resulted in reduced growth and yield. The salinity was proved more devastating than boron toxicity.

In present study, reduction in growth due to the combined salinity and B toxicity was less than the sum of reduction caused by the individual stress factors. Here soil salinity reduced root growth, which in turn decreased the surface area of roots and thus resulted in less uptake of B. It might also be due to antagonistic effect between B and Cl^- at their higher application rates in the presence of salinity. The decrease was attributed to the reason that borates and chlorides, both being anions are absorbed by essentially the same mechanism, excess concentration of Cl^- as found in saline soils may severely affect the absorption of borates because of competitive inhibition (Iqbal *et al*., 2017).

In current experiment, both tested wheat genotypes responded differently to interactive effects of salinity and B toxicity and salt tolerant SARC-I showed better growth exposed to salinity, B and their combined effects. Such tolerance of salinity and B can be due to inherent capacity of the genotype and can contain more tolerant genes to confer

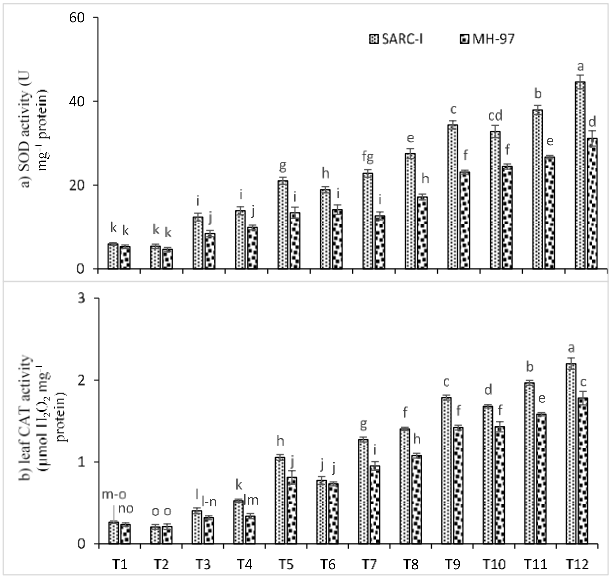

Fig. 4: Antioxidant enzymes (a = leaf superoxide dismutase activity, and b = leaf catalase activity) of sensitive and tolerant bread wheat genotypes in B toxic, saline and B toxic-saline soils (Means ± SE, n = 3)

[LSD for leaf superoxide dismutase activity: V = 0.60*, T = 1.46*, V × T = 2.06*; LSD for leaf catalase activity: V = 0.03*, T = 0.07*, V × T = 0.10*]

[Whereas V = Genotype, T = Treatment, NS = Non-significant ($P > 0.05$), * = Significant ($P \leq 0.05$), ** = Highly significant ($P \leq 0.01$)]

Treatments: T_1 = Control, T_2 = B at 2.5 mg kg^{-1}, T_3 = B at 5 mg kg^{-1}, T_4 = B at 7.5 mg kg^{-1}, T_5 = EC 10 dS m^{-1}, T_6 = EC 10 dS m^{-1} + B at 2.5 mg kg^{-1}, T_7 = EC 10 dS m^{-1} + B at 5 mg kg^{-1}, T_8 = EC 10 dS m^{-1} + B at 7.5 mg kg^{-1}, T_9 = EC 20 dS m^{-1}, T_{10} = EC 20 dS m^{-1} + B at 2.5 mg kg^{-1}, T_{11} = EC 20 dS m^{-1} + B at 5 mg kg^{-1}, T_{12} = EC 20 dS m^{-1} + B at 7.5 mg kg^{-1}

stress (Din *et al*., 2008; Wimmer and Golbach, 2012). The other reason can be differential physiological and biochemical associations of tolerance mechanism and growth of plants (Fariduddin *et al*., 2012).

The physiological parameters such as TCC, PR, TR and SC (Fig. 2) were not significantly affected with 2.5 mg B kg^{-1} in both normal and salt-affected soils. However, the PR in SARC-I was significantly increased at this treatment in normal soil because of the fact that B helps in normal plant growth and in absorption of N, which positively affect the chlorophyll contents and ultimately photosynthesis (Tolanur, 2006). The applied B at greater rates *i.e*., 5 and 7.5 mg kg^{-1} reduced TCC, PR, TR and SC (Fig. 2) in both genotypes both in non-saline and saline condition. 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).

Moreover, salinity as a stressor causes a wide range of physiological changes such as disruption of membranes, diminished mineral nutrition, damages the ability to detoxify ROS, alterations in the antioxidant enzymes and reduced photosynthetic activity by decreasing SC, TR and biosynthesis of photosynthetic pigments (Gupta and Huang, 2014), all these modifications results in severe reduction in both dry biomass and crop yields (Iqbal *et al*., 2015).

Salinity and B toxicity in combination, affect photosynthesis in plants by disrupting the photosynthetic pigment's biosynthesis and also affect membrane functions (Karabal *et al*., 2003).

In consonance with the present study results, the reduction in leaf B (Fig. 3a) with the increase of NaCl concentration has been widely reported. This might be due to the reason that B and Cl^- are absorbed by essentially the same mechanism; excess concentration of Cl^-, as found in highly saline soils, may severely affect the uptake of borates because of competitive inhibition. Another reason is the reduced rates of transpiration limiting the leaf accumulation of B that is transported through the xylem (Yermiyahu *et al*., 2008). Smith *et al.* (2010) found that at low level of B, the increasing salinity increased shoot B concentration in broccoli. However, at higher level of B, the increased salinity decreased shoot B concentration. Salinity reduced B uptake by plants but the impact was mainly evident at high substrate B concentrations, an observation also found in wheat by Wimmer and Goldbach (2012). Similarly, with increasing B level, decrease in leaf Cl^- was due to the enhanced completion with borate ions for uptake. Lee (2006) also reported reduced Cl^- in hot pepper leaves in the presence of combined B toxicity and salinity than salinity alone. It is possible that B exporters also export the other anions such as Cl^- along with B as a side effect and thus the concentration of Cl^- is reduced in response to B toxicity.

In present study, it has been observed that the leaf Na^+ concentration increased (Fig. 3b) while K^+ concentration (Fig. 3c) decreased with increasing soil salinity. Moreover, the increasing B had no impact on leaf Na^+ and while increased leaf K^+ concentration. The results of present experiment were also supported by previous findings. Under salt stress, the activity of specific membrane components can be influenced by B regulating the functions of certain aquaporin isoforms as possible components of the salinity tolerance mechanism (Martinez-Ballesta *et al*., 2008).

The results showed that salt tolerant and sensitive wheat genotypes responded differently to combine salinity and B toxicity, showing high B concentration in MH-97 compared to SARC-I. Reid and Fitzpatrick (2009) reported that B tolerant wheat genotypes relieved B toxicity in leaves and roots by means of B exporters expressed in both roots and leaves. Moreover, at the plasma membrane, boric acid channels have been demonstrated to be in involved in maintenance of low B concentrations inside the cells (Miwa and Fujiwara, 2010). The mechanisms for B tolerance are similar to those described for salt tolerance, and plant species vulnerable to excess B, accumulated more B in leaves and stems than tolerant species (Nable *et al*., 1997).

The results of current study showed that tolerant wheat genotype SARC-I maintained higher leaf K^+ and lower leaf Na^+ concentration than the sensitive MH-97. The enhanced uptake of K^+ in leaves of tolerant genotype resulted in high K^+/Na^+ ratio which is beneficial for cellular homeostasis.

Production of excess level of reactive oxygen species (ROS) is a general phenomenon in a plant under stress condition during the normal course of metabolism. Oxidative stress has been reported under both B-deficiency (Han *et al*., 2008) and B-excess (Molassiotis *et al*., 2006) in different plant species. The chloroplasts and mitochondria of plant cells are important intracellular generators of ROS. Electrons leaked from electron transport chains can react with O_2 during normal aerobic metabolism to produce ROS such as superoxide, hydrogen peroxide, and the hydroxyl radical (Thompson *et al*., 1987). The ROS and the radicals derived from ROS are highly bioactive and cause cellular damages in plants (Zhang *et al*., 2011). The SOD and CAT are considered as the front line defense antioxidant enzymes that detoxify the ROS and consequently reduce the cellular damage in plants. In present pot study the activity of SOD and CAT (Fig. 4) had significantly ($P \leq 0.05$) affected by soil salinity, B and their interaction. The activity of SOD and CAT increased gradually with increasing B and soil salinity. The tolerant wheat genotype had higher activity of both enzymes than the sensitive one. The present results were in agreement with the previous reports. Hossain *et al.* (2015) found that the application of 4.5 mg B kg^{-1} soil improved the activities of antioxidant protective enzyme of SOD in tested rapeseed cultivars. In another study, Eraslan *et al.* (2007) reported that when carrot plants were subjected to salinity and excess B simultaneously, the activity of antioxidant enzymes like CAT was increased. Similarly, lipid peroxidation and the activities of CAT and SOD were increased in grapevine in response to combined stresses of high B and salts. Increased H_2O_2 indicate membrane damage caused by oxidative stress and as a result membrane permeability increases under excess B and salinity (Soylemezoglu *et al*., 2009). It has been established that stress tolerant genotypes exhibited higher antioxidant enzyme activities (Sairam and Srivastava, 2002). The tolerance of plants might be associated with increased capacity of the antioxidative system (total SOD and CAT) to scavenge ROS and thus reduce lipid peroxidation under stress conditions (Ardic *et al*., 2009). Thus, the enzymatic activities of the antioxidant system in plants under stress can be considered as an indicator of tolerance of genotypes against stress condition.

Conclusion

In present study, growth, yield and physiological responses of tolerant and sensitive wheat genotypes were significantly ($p \leq 0.05$) affected by applied NaCl salinity and B treatments individually as well as in combination. The tolerant (SARC-I) and sensitive (MH-97) wheat genotypes showed significant ($p \leq 0.05$) variations to NaCl salinity and B stress, Na^+ and K^+ accumulation, antioxidant enzymes (*i.e.*, SOD and CAT), plant growth and physiological functions. The soil salinity at 20 dS m^{-1} had highly devastating effects on wheat growth and yield. The interaction between salinity and toxic B levels *i.e*., 5 and 7.5 mg kg^{-1} soil, was found antagonistic considering their impact on plant growth and physiological processes. The salt-tolerant SARC-I wheat genotype showed tolerance to B toxicity as well as combined salinity and B stresses, hence can be used in future breeding programs or can be grown by farmers under B-toxic, saline or B-toxic saline environment. Moreover, the present results revealed that adequate level of B fertilization (2.5 mg kg^{-1} soil) partially decreased the harmful effects of salinity and promoted wheat growth both under non saline and saline conditions by alleviating antioxidant stress and uptake of Cl^- ions by plants.

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(Received 04 November 2017; Accepted 16 November 2018)