

Physiological responses to excess boron in wheat cultivars

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ABSTRACT

This study investigates the response of two wheat cultivars to boron toxicity stress. Plants were cultivated in sand culture and boron was applied to the culture for 10-day. Symptoms, tiller number, boron concentration, soluble sugars, proteins and other free amino acids than proline were studied. The differences between the cultivars were apparent from higher boron and the chlorosis in tolerant cultivar was about 7% compared to the sensitive one 70%. Tiller number gradual decreased in tolerant-cultivar, while in sensitive one a dramatic reduction was exhibited by increasing boron level in culture media. In most boron levels, although the accumulation of soluble carbohydrates was significantly stimulated in shoot of B-sensitive cultivar (Gemmeza 9; S), there were no appreciable differences in the production of carbohydrates in shoot of B-tolerant cultivar (Sakha 93; T). However, the soluble proteins production did not affect by most boron levels in both cultivars. The presence of boron at various concentrations induced a production of free amino acids in shoots of each of the two test cultivars. Tiller number (yield index) decreased in the two test cultivars and was in range 50-59 and 84-92% less than control plants for tolerant and sensitive cultivar, respectively.

Keywords: Amino acids; Boron; Pigments; Soluble carbohydrates; Soluble proteins.

1. INTRODUCTION

Boron (B) is well documented as an essential micronutrient for optimum growth of vascular plants. However, when B is present above the permissible limit in the soil or ground water, plant growth and reproduction can be affected, limiting crop productivity throughout the world [1, 2]. Boron toxicity is extensively located in the agricultural areas of Australia, North Africa, and West Asia characterized by alkaline and saline soils together with a low rainfall and very scarce leaching. In addition to this, B-rich soils also occur as a consequence of over fertilization and/or irrigation with water containing high levels of B [3, 4].

Negative impacts of excess B involves many developmental/biochemical processes in plants such as altered metabolism [5, 6], reduced activity in photosynthetic process [7], reduced root cell division [8], reduced shoot cell wall expansion [5] and generation of reactive oxygen species (ROS) followed by oxidative damage [9, 10]. Reid et al. [2] also demonstrated that excess B impairs the tolerance to photo-oxidative stress.

Boron is unique as a micronutrient: it has restricted mobility in many plant species while it is freely mobile in others [11]. Boron mobility within

plant parts determines the visible symptoms of B excess: in plants with low B mobility, the typical symptoms are chlorotic and/or necrotic patches (burn) of the older leaves where B tends to accumulate [12]. Differently, in plants with high B mobility the symptoms of B toxicity firstly appear in meristematic regions and in fruits, but not in mature leaves [13]. Moreover, B toxicity can affect crop productions through the reduction of leaf expansion, photosynthetic efficiency and fruit set [12]. The ability to restrict B uptake into the plants can minimize the physiological impairments caused by B toxicity [12, 14]. On the other hand, an inherent ability to tolerate excessive B concentration in plant tissues [15] or the differential antioxidant response that may reduce B-toxicity damage in some species [10] was suggested.

We are studying the toxicity effects of B on wheat cultivars and have earlier shown that Sakha 93 the most B-tolerant, out of five, test cultivars and Gemmeza 9 as the most B-sensitive one [16]. Thus, in this investigation it seemed necessary to consider some physiological and biochemical responses of the selected cultivars and how far these responses are correlated with the B-tolerance mechanisms at different B levels. Particular attention was focused to investigate the correlations among the B, toxicity symptoms, yield index, soluble carbohydrates, soluble proteins and other free amino acids than proline concentrations in the plant tissues of both the cultivars.

2. MATERIALS AND METHODS

2.1. Plant material, growth conditions, and treatments

Seeds of B-sensitive (Gemmeza 9; S) and B-tolerant (Sakha 93; T) cultivars of wheat (*Triticum aestivum* L.) were sterilized and grown in sand culture in 10 cm diameter plastic pots lined with polyethylene bags [16]. Fifteen grains were grown in 0.7 kg air-dried and cleaned quartz sand, which was kept at approximately 100% of the field capacity by watering with B-free distilled water and left for germination in a greenhouse under natural light. After 10 days, 5 seedlings were selected on the basis of vigor and uniformity, the undesired seedlings were removed. Then, boron stress treat-

ment was initiated by applying Nable's solution [17] containing boric acid (H_3BO_3) to the seedlings, the pH was buffered to pH 5.7. The seedlings were grown in final B concentration of: 1, 3, 6, 8 and 10 $mg\ kg^{-1}$ soil for ten days for vegetative growth and 35 days for tiller stage. Each pot represents as experimental unit with 5 plants per treatment; each treatment was replicated six times. The samples were collected: roots and shoots separated, washed with deionized water, weighed, frozen in liquid nitrogen, and stored at $-80^\circ C$ and some samples were oven-dried at $70^\circ C$ for 48 hours.

2.2. Plant extraction

Shoot extractions were prepared using 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 g polyvinylpyrrolidone (PVP) and used for the determination of soluble carbohydrates, soluble protein and other free amino acids than proline.

2.3. Soluble carbohydrates

Phosphate buffer extraction was mixed with anthrone reagent [18, 19]. The samples were placed in a boiling water bath for 10 min. The light absorption of the samples was determined spectrophotometrically at 625 nm. A calibration curve using pure glucose was constructed.

2.4. Soluble proteins

Proteins in the extract were estimated by Folin Ciocalteu's reagent [20]. The absorbance of color was measured using a spectrophotometer at 750 nm. A calibration curve was constructed using bovine serum albumin (BSA).

2.5. Amino acids

Other free amino acids than proline were determined using ninhydrin [21] and were measured using a spectrophotometer at 570 nm. A calibration curve was constructed using glycine.

2.6. Boron concentration

For boron concentration measurement, 0.01 g (DW) shoot samples were dry ashed in a muffle

furnace at 500°C for 6 h. The ash was then dissolved in 0.1 N HCl and B was determined colorimetrically at 540 nm by the curcumin method [22].

2.7. Statistical analysis

All measurements were taken in independent 6 replications. The data given in all figures represent means \pm SE. Statistical assays were carried out by using ANOVA (completely randomized) to determine if significant differences were present among means. Duncan's test was carried out to determine if mean difference significant at $P \leq 0.05$ (SPSS-11).

3. RESULTS

The response of plants to toxic levels of B has received renewed interest of late. There is a wealth of information about the effects of B toxicity on the biomass parameters and the metabolic response of plants corresponding to respective B concentrations in the growth medium. This large set of data was difficult to treat and present in full and thus simple two-dimensional parameter correlations are presented and discussed in the text below.

The main concern is that B is mainly transported *via* the transpiration stream and accumulated in leaves, whereas B cannot be remobilized in wheat plants. Therefore, all the studied parameters have done on leaves of two wheat cultivars (Gemmeza 9 and Sakha 93, the B-sensitive and B-tolerant cultivars, respectively).

3.1. Correlation between B and toxicity symptom

The symptoms reflect the distribution of B in most species with B accumulating at the end of the transpiration stream. The current study showed that wheat is very sensitive to excess B and has a relatively low B-demand during vegetative growth, accompanied by a high susceptibility to B toxicity. When the B concentration in soil was exceeded 1 mg B kg⁻¹ soil characteristic symptoms of B toxicity appeared (Fig. 1). The first sign of B toxicity was yellow-green chlorosis, which first developed on the oldest leaves and progressed to the youngest. Later, small patches of necrotic tissue appeared. In Gemmeza 9, the B-sensitive cultivar,

the chlorotic symptoms appeared 3 days after treatments, while in Sakha 93, the B-tolerant cultivar; symptoms appeared 7 days after treatments.

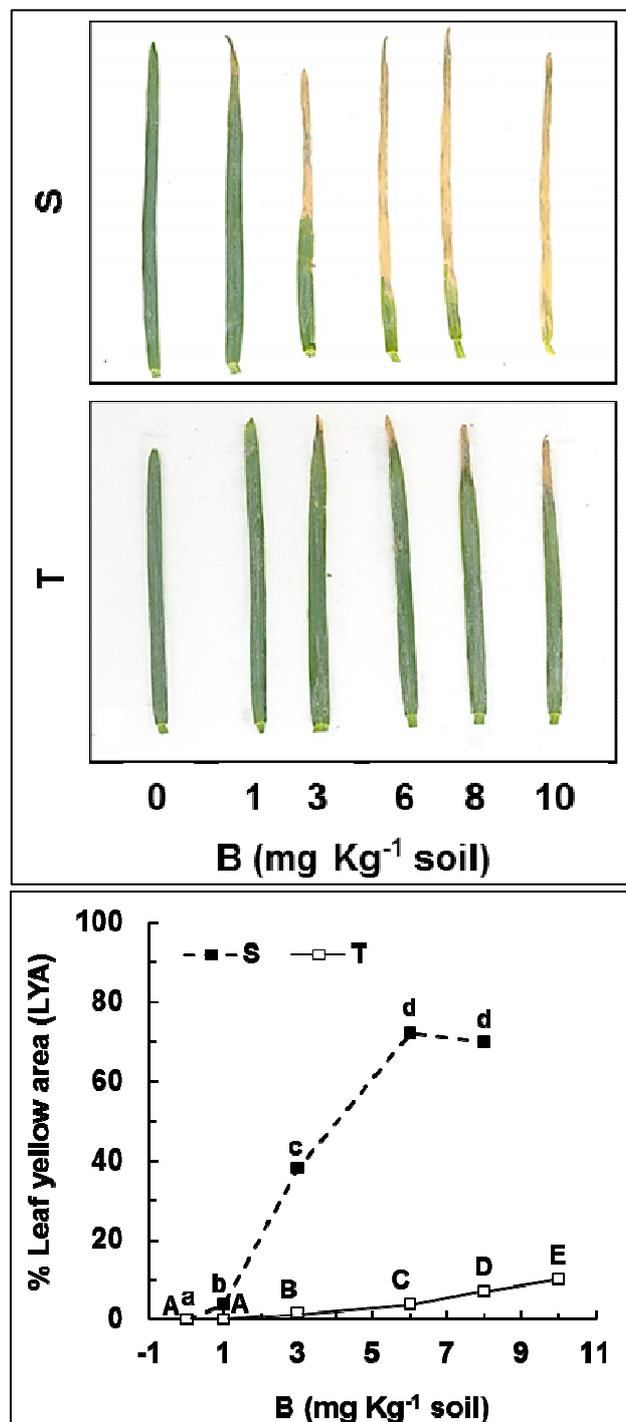


Figure 1. Dependence of leaf symptoms of 20-day-old Gemmeza 9 (S) and Sakha 93 (T), the B-sensitive and B-tolerant wheat cultivars, respectively, as affected by different boron levels in sand soil for 10 days. Yellow areas are expressed in terms of % of the leaf area. Data points represent mean \pm standard error ($n = 6$).

3.2. Correlation between B content and tiller number

Special emphasis was laid on the influence of B toxicity stress on tiller number (yield index) of the two test wheat cultivars. In this respect, considerable differences were induced by the various levels of B (Figs. 2 and 3). The results presented in Figure 2 reveal that the tiller number of 35-day-old plants was markedly affected by B level. Tiller number decreased in the two test wheat cultivars (sensitive and tolerant) as B level increased. At 1, 3, and 6 mg B kg⁻¹ soil the reduction in tiller number of Gemmeza 9 was quit pronounced (50%, 84%, and 92%, respectively) as compared with control. However, the decrease in tiller number of Sakha 93, the B-tolerant cultivar, at 3 and 6 mg B kg⁻¹ soil B level amounted to about 50% and 59%, respectively as compared with control.

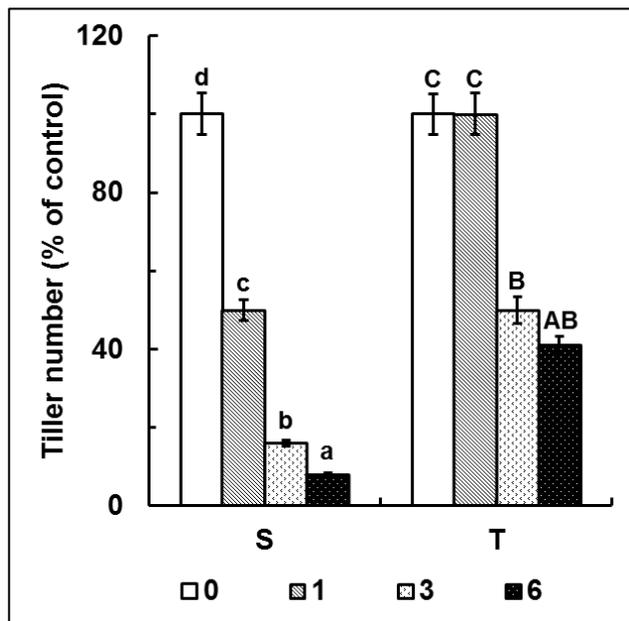


Figure 2. Tiller number (% of control) of 35-day-old of Gemmeza 9 (S) and Sakha 93 (T), the B-sensitive and B-tolerant wheat cultivars respectively, supplemented with different boron concentrations. Data points represent mean ± standard error (n = 6).

The data in Fig. 4 clearly demonstrated that B concentration in shoots of Sakha 93 and Gemmeza 9 increased gradually with the rise of B level in the soil. It is worth to mention that significant differences in B concentrations in shoots of Sakha

93 and Gemmeza 9 cultivars were manifested. At low B concentrations (1, 3 mg B kg⁻¹ soil) Sakha 93 had B concentrations in shoots from 25-to 29% greater than in sensitive cultivar Gemmeza 9, while at higher levels (6, 8 mg B kg⁻¹ soil) Gemmeza 9 had B concentrations in shoots from 33.5 to 55% greater than in Sakha 93.

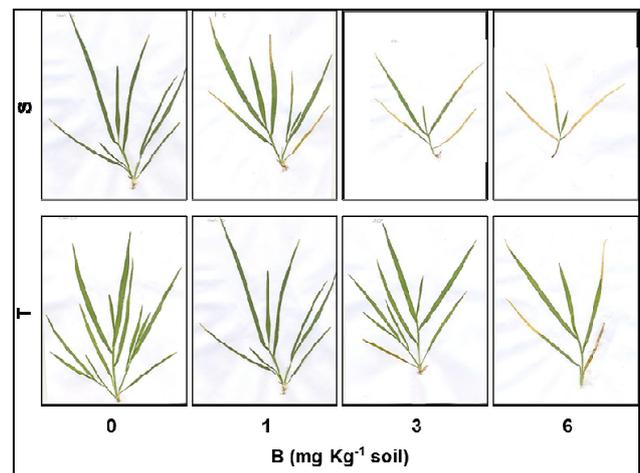


Figure 3. Tillering of 35-day-old of Gemmeza 9 (S) and Sakha 93 (T), the B-sensitive and B-tolerant wheat cultivars respectively, supplemented with different boron concentrations.

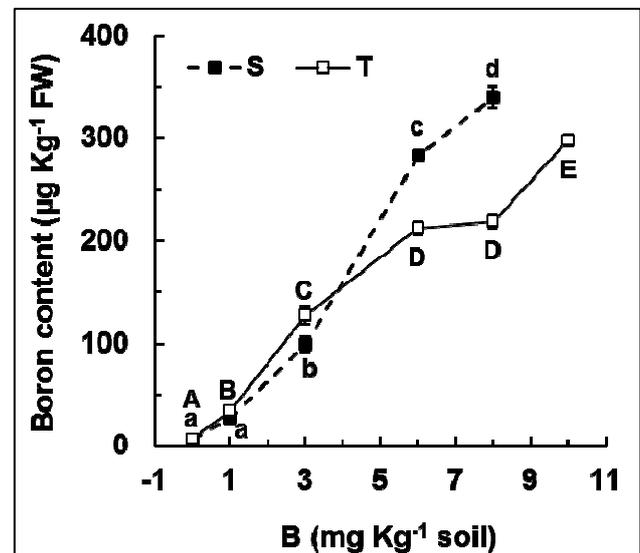


Figure 4. Shoot boron contents of 20-days-old Gemmeza 9 (S) and Sakha 93 (T), the B-sensitive and B-tolerant wheat cultivars respectively, as affected by different boron levels in sand soil for 10 days. Data points represent mean ± standard error (n = 6).

3.3. Correlation between B and soluble metabolites

The response of the tissue soluble sugars, proteins and amino acids concentrations toward B accumulation was variable in shoots of the tolerant cultivar and sensitive one (Fig. 5).

Although the accumulation of soluble carbohydrates was significantly stimulated in shoot of Gemmeza 9 with the increase of most B level, there were no appreciable differences in the production of carbohydrates in shoot of Sakha 93 at most B levels (Fig. 5A).

The successive increase in B concentration did not induce a stimulatory effect on the accumulation of soluble proteins in the shoots of the two test cultivars, except at level 6 mg B kg⁻¹ soil, which was of a stimulatory effect on the synthesis of proteins in shoots of Sakha 93 (Fig. 5B).

The results presented in Figure 5C clearly demonstrate that the presence of B in the culture media at various concentrations induced appreciable induction in the production of other free amino acids than proline in shoots of each of the two test cultivars.

4. DISCUSSION

4.1. Correlation between B and toxicity symptom

The common feature of tolerant cultivars was that the B concentrations in their tissues were lower than in sensitive cultivars. From this, it was hypothesized that the tolerance trait was associated with an ability to restrict B uptake from the soil into the roots, thereby reducing transfer to the shoot [23]. The first visible result obtained in this experiment was the different sensitivity to B stress shown by the two wheat cultivars evaluated. In fact, marginal portion of leaves exhibited evident and marked symptoms of damage in Gemmeza 9, whereas these were scarce in Sakha 93. These symptoms which represent the general symptoms of B toxicity reflect the distribution of B in most species, with B accumulating at the end of the transpiration stream [24, 25]. Kohl and Oertli [26] demonstrated that B uptake followed the passive water flux from roots to leaves accumulated especially where termination of leaf veins terminate; these tissues show more

evident symptoms of B toxicity such as chlorosis and necrosis. According to Shelp [27] higher B concentrations were found in leaf tissues than in phloem sap.

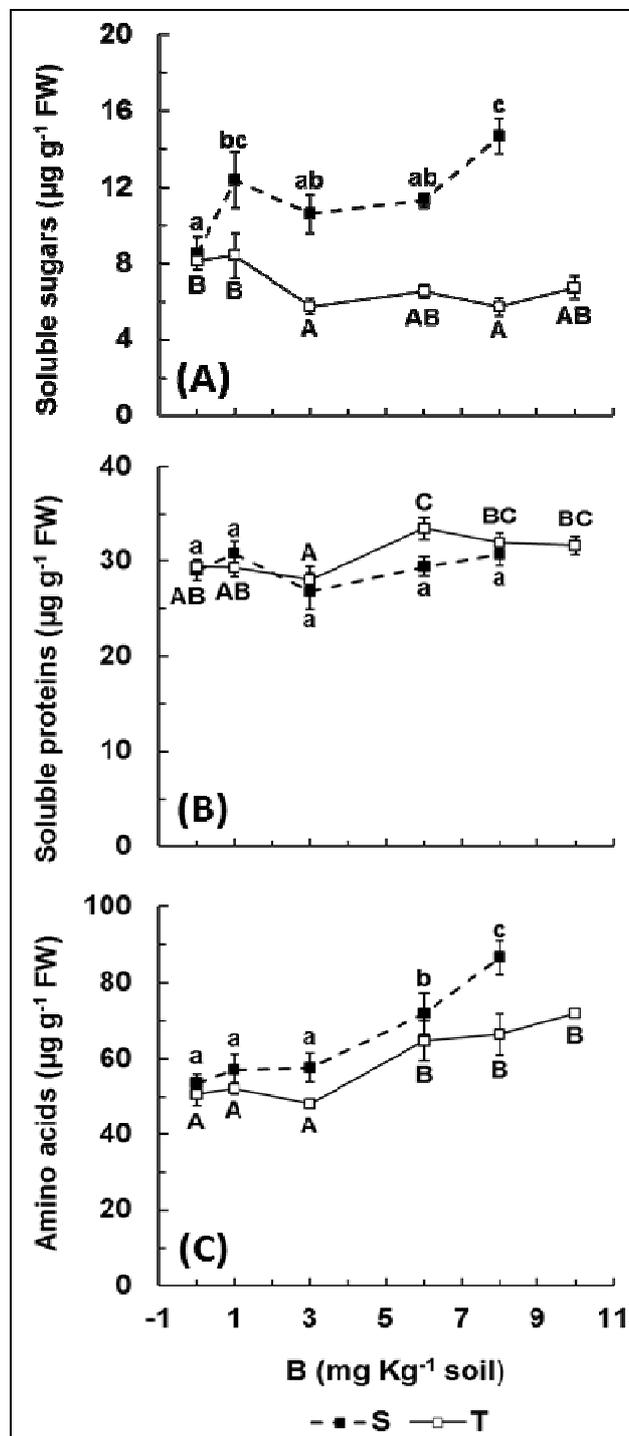


Figure 5. Soluble sugars (A), soluble proteins (B) and amino acids (C) concentration in shoot of 20-day-old Gemmeza 9 (S) and Sakha 93 (T), the B-sensitive and B-tolerant wheat cultivars respectively, as affected by different boron levels in sand soil for 10 days. Data points represent mean \pm standard error (n = 6).

4.2. Correlation between B content and tiller number

Growth and yield were reported to be limited in all cases where plants were grown under root zone conditions of high B [28]. It is known that presence of high amount of B in irrigation water and soil adversely affects plant growth and yield production in different cereal plants [16, 29] due to its ease in absorption and mobility within plant cell/tissues [12, 30].

Tillering or vegetative branching is one of the most important components of shoot architecture in cereals because it contributes directly to grain yield [31, 32] and is involved in plant plasticity in response to environmental cues and stresses [33, 34]. In this study, the tiller number of both test cultivars decreased with increasing B level in the soil. This decrease was more evident in the sensitive cultivar where the B of only 1 mg kg⁻¹ soil decreased the tiller number to about 50% of the control weight. B toxicity impacts heavily on wheat production in Australia (up to 11% yield reduction in affected areas [35], and breeding for tolerance in wheat is of high importance across southern Australia.

Species and genotypes susceptible to B toxicity generally have higher concentrations of B in leaves and shoots than do tolerant genotypes [36]. Gemmeza 9 is more susceptible to B toxicity and accumulates more B in its shoots than Sakha 93, the B tolerant cultivar. Working on several barley and wheat genotypes, Nable [17] reported that the most susceptible genotypes to excess B accumulate more B than tolerant genotypes. Some authors recorded a range of genotypic variation in response to B toxicity with mechanisms including B exclusion [37, 38] and an inherent ability to tolerate excessive B concentration in plant tissues [15]. It was observed that the B-tolerant barley cultivar Sahara 3771 has the capacity to maintain much lower B concentrations in roots as well as in xylem and leaves [14], for which the authors propose a mechanism of active efflux of the borate anion.

4.3. Correlation between B and soluble metabolites

Boron plays a key role in sugar transport and carbohydrate metabolism [39]. Our results demon-

strate that B toxicity resulted in increased soluble carbohydrates in the shoot of Gemmeza 9. Under similar conditions there was no appreciable change in soluble carbohydrates in Sakha 93. The carbohydrate accumulation seems to be related to limitation of its use rather than increase in its synthesis. Cervilla et al. [10] reported that B-toxicity increased glucose, fructose and sucrose contents in the leaves of two tomato (*Lycopersicon esculentum*) cultivars ('Josefina' and 'Kosaco'). Pérez-López et al. [40] reported that the accumulation of sugars in plants under stress conditions might be involved in the osmotic adjustment. However, the protective role of sucrose could be explained as a compatible solute, protecting structure of membranes [41].

The results of the present work demonstrate that B failed to induce appreciable variations in the production of soluble proteins in shoots of the two test cultivars. This result agrees with the results reported by Reid et al. [2] who demonstrated that neither photosynthesis, respiration nor protein synthesis was particularly sensitive to B. Also, Uluisik et al. [42] showed that boron treatment does not change the expression pattern of most of the ribosomal protein genes.

Amino acids acts as a putative osmoprotective solute leading to lowering osmotic potential in several tissues exposed to stress [43]. In our experiment, both wheat cultivars subjected to high B toxicity (up 3 mg/kg soil) showed a significant increase in the content of amino acids. This observation is in accordance with that of Gopal [44] who indicated that application of 10 ppm B in sand culture was highly injurious to groundnut plant. The chlorotic leaves showed decreases in protein-nitrogen and considerable increase in soluble-nitrogen, contents of aspartic acid, glutamic acid, glycine and alanine. Also, Kaya et al. [45] suggested that B stress induces amino acid synthesis or activates the general amino acid control mechanism.

5. CONCLUSIONS

In conclusion, *T. aestivum* accumulates B in the oldest leaves and progresses to the youngest. The differences between the cultivars were statistically apparent only at 3 mg kg⁻¹ soil levels of B

accumulation. Tiller number decreased in the two test cultivars and was found in the range from 50-59 and 84-92% less than control plants at 3 and 6 mg B kg⁻¹ soil for tolerant and sensitive cultivars, respectively. The soluble carbohydrates and proteins concentrations did not affect by B accumulation, except for the carbohydrates of the sensitive cultivar where the concentration was stimulated. Both wheat cultivars subjected to B up to 3 mg kg⁻¹ soil showed a significant increase in the content of amino acids.

AUTHORS' CONTRIBUTIONS

AMM and AMH conceived and designed research. AMM and RME conducted experiments. AMM, RME and AMH analysed data and wrote the manuscript. All authors read and approved the final manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

REFERENCES

1. Stangoulis JC, Reid RJ. Boron toxicity in plants and animals. In: Boron in plant and animal nutrition. Goldbach H, Rerkasem B, Wimmer M, Brown PH, Thelier M, Bell R, eds. Kluwer, Dordrecht, Netherlands, 2002: 227-240.
2. Reid RJ, Hayes JE, Post A, Stangoulis JC, Graham RD. A critical analysis of the causes of boron toxicity in plants. *Plant Cell Environ.* 2004; 27: 1405-1414.
3. WHO. Guidelines for Drinking-water Quality, 2nd ed. Addendum to vol 2: Health Criteria and Other Supporting Information. World Health Organization, Geneva, (WHO/EOS/98.1), 1998.
4. Parks JL, Edwards M. Boron in the Environment. *Crit Rev Env Sci Tec.* 2005; 35: 81-114.
5. Loomis WD, Durst RW 1992. Chemistry and biology of boron. *BioFactors.* 1992; 3: 229-239.
6. Cervilla LM, Blasco B, Ríos JJ, Rosales MA, Rubio-Wilhelmi MM, Sánchez-Rodríguez, E, et al. Response of nitrogen metabolism to boron toxicity in tomato plants. *Plant Biol.* 2009; 11: 671-677.
7. Han S, Tang N, Jiang HX, Yang LT, Li Y, Chen LS. CO₂ assimilation, photosystem II photochemistry, carbohydrate metabolism and antioxidant system of citrus leaves in response to boron stress. *Plant Sci.* 2009; 176: 143-153.
8. Konuk M, Liman R, Cigerci IH. Determination of genotoxic effect of boron on *Allium cepa* root meristematic cells. *Pak J Bot.* 2007; 39: 73-79.
9. Molassiotis A, Sotiropoulos T, Tanou G, Diamantidis G, Therios I. 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.* 2006; 56: 54-62.
10. Cervilla LM, Blasco B, Ríos JJ, Romero L, Ruiz JM. Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to boron toxicity. *Ann Bot.* 2007; 100: 747-756.
11. Brown PH, Shelp BJ. Boron mobility in plants. *Plant Soil.* 1997; 193: 85-101.
12. Nable RO, Bañuelos GS, Paull JG. Boron toxicity. *Plant Soil.* 1997; 193: 181-198.
13. Brown PH, Hu H. Phloem mobility of boron is species dependent: evidence for phloem mobility in sorbitol-rich species. *Ann Bot.* 1996; 77: 497-506.
14. Hayes JE, Reid RJ. Boron tolerance in barley is mediated by efflux of boron from the roots. *Plant Physiol.* 2004; 136: 3376-3382.
15. Torun AA, Yazici A, Erdem H, Cakmak I. Genotypic variation in tolerance to boron toxicity in 70 durum wheat genotypes. *Turk J Agric For.* 2006; 30: 49-58.
16. Metwally A, El-Shazoly R, Hamada AM. Effect of boron on growth criteria of some wheat cultivars. *J Biol Earth Sci.* 2012; 2(1): B1-B9.
17. Nable RO. Resistance to boron toxicity amongst several barley and wheat cultivars: a preliminary examination of the resistance mechanism. *Plant Soil.* 1988; 112: 45-52.
18. Fales FW. The assimilation and degradation of carbohydrates by yeast cells. *J Biol Chem.* 1951; 193: 113-124.
19. Schlegel HG. Die verwertung organischer säuren durch *Chlorella* in licht. *Planta.* 1956; 47: 510-526.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem.* 1951; 193: 265-275.
21. Moore S, Stein WH. Photometric ninhydrin method for use in the chromatography of amino acids. *J Biol Chem.* 1948; 176: 367-388.
22. Jackson ML. Soil chemical analysis. Constable and Co. Ltd. Prentice Hall of India Pvt. Ltd. New Delhi, 1973: 10-114.
23. Reid R. Understanding the boron transport network in plants. *Plant Soil.* 2014; 385: 1-13.

24. Bergmann W. Colour atlas: nutritional disorders of plants. Gustav Fischer, New York, USA, 1992: 204-239.
25. Bennett WF. Nutrient deficiencies and toxicities in crop plants. APS Press, St. Paul, Minnesota, USA, 1993.
26. Kohl HC, Oertli JJ. Distribution of boron in leaves. *Plant Physiol.* 1961; 36: 420-424.
27. Shelp BJ. Boron mobility and nutrition in broccoli (*Brassica oleracea* var. *italica*). *Ann Bot.* 1988; 61: 83-91.
28. Choi EY, Kolesik P, McNeill A, Collins H, Zhang Q, Huynh BL, et al. The mechanism of boron tolerance for maintenance of root growth in barley (*Hordeum vulgare* L.). *Plant Cell Environ.* 2007; 30: 984-993.
29. El-Feky SS, El-Shintinawy FA, Shaker EM. Role of CaCl₂ and salicylic acid on metabolic activities and productivity of boron stressed barley (*Hordeum vulgare* L.). *Int J Curr Microbiol Appl Sci.* 2014; 3: 368-380.
30. Reid R. Can we really increase yields by making crop plants tolerant to boron toxicity? *Plant Sci.* 2010; 178: 9-11.
31. Kebrom TH, Spielmeier W, Finnegan EJ. Grasses provide new insights into regulation of shoot branching. *Trends Plant Sci.* 2013; 18: 41-48.
32. Hussien A, Tavakol E, Horner DS, Muñoz-Amatriaín M, Muehlbauer GJ, Rossini L. Genetics of tillering in rice and barley. *Plant Genome.* 2014; 7: 1-20.
33. Mohapatra PK, Panda BB, Kariali E. Plasticity of tiller dynamics in wild rice *Oryza rufipogon* Griff.: a strategy for resilience in sub-optimal environments. *Int J Agron.* 2011; 9: 543237.
34. Agusti J, Greb T. Going with the wind - adaptive dynamics of plant secondary meristems. *Mech Develop.* 2013; 130: 34-44.
35. Moody DB, Rathjen AJ, Cartwright B. Yield evaluation of a gene for boron tolerance using backcross-derived lines. In: Randall P, Delhaize E, Richards RA, Munns R, eds. Genetic aspects of plant mineral nutrition. Kluwer Academic Publ, Dordrecht, Netherlands, 1993: 363-366.
36. Nable RO, Banuelos GS, Paull JG. Boron toxicity. *Plant Soil.* 1997; 198: 181-198.
37. Nable RO, Cartwright B, Lance RC. Genotypic differences in boron accumulation in barley: Relative susceptibilities to boron deficiency and toxicity. In: Genetic aspects of plant mineral nutrition. Bassam NE, Damborth M, Laoghman B, eds. Kluwer Academic Publishers, Dordrecht, Boston, 1990: 243-251.
38. Jefferies SP, Barr AR, Karakousis A, Kretschmer JM, Manning S, Chalmers KJ, et al. Mapping of chromosome regions conferring boron toxicity tolerance in barley (*Hordeum vulgare* L.). *Theor Appl Gen.* 1999; 98: 1293-1303.
39. Dugger WM. Boron in plant metabolism. In: Lauchli A, Bielecki RL, eds. Encyclopedia of plant physiology. Springer-Verlag, Berlin, 1983; 15B: 626-650.
40. Pérez-López U, Robredo A, Lacuesta M, Muñoz-Rueda A, Mena-Petite A. Atmospheric CO₂ concentration influences the contributions of osmolyte accumulation and cell wall elasticity to salt tolerance in barley cultivars. *J Plant Physiol.* 2010; 167(1): 15-22.
41. Pastorczyk M, Giełwanowska I, Lahuta LB. Changes in soluble carbohydrates in polar Caryophyllaceae and Poaceae plants in response to chilling. *Acta Physiol Plant.* 2014; 36: 1771-1780.
42. Uluisik I, Kaya A, Fomenko DE, Karakaya HC, Carlson BA, Gladyshev VN, Koc A. Boron stress activates the general amino acid control mechanism and inhibits protein synthesis. *PLoS One.* 2011; 6(11): e27772.
43. Rady MM, Sadak MS, El-Bassiouny HM, Abd El-Monem AA. Alleviation the adverse effects of salinity stress in sunflower cultivars using nicotinamide and α-tocopherol. *Aust J Basic Appl Sci.* 2011; 5: 342-355.
44. Gopal NH. Effect of excess boron supply on accumulation of boron and nitrogen metabolism in groundnut plant (C. F.). *Soil Fert.* 1971; 34: 5334-5340.
45. Kaya M, Herniou EA, Barraclough TG, Fontaneto D. Inconsistent estimates of diversity between traditional and DNA taxonomy in bdelloid rotifers. *Org. Divers. Evol.* 2009; 9: 3-12.