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BORON TOXICITY ALLEVIATION BY ZINC APPLICATION IN TWO BARLEY CULTIVARS DIFFERING IN TOLERANCE TO BORON TOXICITY

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Zinc (Zn) deficiency and boron (B) toxicity are often encountered simultaneously in soils of semi-arid and arid regions. A sand culture experiment was conducted to evaluate the alleviation of B toxicity by application of Zn to barley genotypes. Two barley cultivars [Clipper (B toxicity sensitive/Zn-inefficient) and Sahara (B toxicity tolerant/Zn-efficient)] were grown with four B (0.1, 0.5, 1.5 or 3 mg kg^{-1}) and two Zn (0.1 or 1.0 mg kg^{-1}) levels, for 35 days. High rates of B significantly reduced plant dry matter, more prominently in Clipper. Application of Zn significantly improved shoot and root dry matter in Clipper in all B treatments. Boron application significantly increased B in shoots and roots of both genotypes, but the increase was more prominent in Clipper than Sahara. Boron concentration in roots of Clipper was almost double that in Sahara irrespective of Zn and B treatments. Application of Zn at 3.0 mg Zn kg^{-1} decreased B concentration in both cultivars. Higher Zn concentration in Sahara resulted in lower B:Zn concentration ratio than Clipper. The interactive effects of B and cultivars suggested the possible use of genetic variations for ameliorating B toxicity and Zn deficiency.
Keywords: Abiotic stresses, arid regions, mineral nutrition, nutrient interaction

INTRODUCTION

Boron (B) is an important micronutrient for higher plants and its deficiency leads to reduced yield and quality of crops (Marschner, 1995; Kanwal *et al.*, 2008). The threshold concentration between deficiency and toxicity of B is very narrow (Mortvedt *et al.*, 1991). In terms of hot-water-soluble B in soils, less than 0.5 mg kg^{-1} may cause deficiency and more than 5 mg B kg^{-1} may cause phytotoxicity (Rashid & Ryan, 2004).

Boron toxicity results in changed metabolism, reduced cell division, lower photosynthetic rates, decreased leaf chlorophyll content, lignin and suberin concentrations (Roessner *et al.*, 2006; Reid 2010) resulting in reduced growth of shoots and roots (Nable *et al*. 1997). Boron toxicity is a major issue particularly in alkaline and salt-affected soils in low-rainfall areas (Marschner, 1995; Nable *et al.*, 1997). Boron toxicity in soils may also be caused by irrigation with water containing high concentrations of B and or over fertilization (Nable *et al.*, 1997; Tanaka and Fujiwara, 2008). Reclamation of B-toxic soils is difficult in most cases (Reid, 2010). Therefore, selecting or breeding for increased B toxicity tolerance in plants is a practical approach to increase or maintain yields on high B-soils (Yau and Ryan, 2008).

Genetic variation for B toxicity tolerance exists in a number of crop species, including barley (*Hordeum vulgare* L.) (Roessner *et al*., 2006; Miwa *et al*. 2006; Hayes and Reid, 2004; Reid and Fitzpatrick, 2009). Barley is grown in a wide range of climates, facing B toxicity mainly in areas with alkaline soils and low rainfall. Barley genotype Sahara 3771 (hereafter referred to as Sahara), an Algerian land race, has high tolerance to B toxicity by extruding B from roots (Hayes and Reid, 2004; Roessner *et al.*, 2006). Exploitation of this genetic diversity for B toxicity tolerance in barley may significantly improve both the productivity and yield in areas liable to the B-toxicity problem.

Zinc (Zn) deficiency is the most widespread micronutrient deficiency in cereals around the globe, causing reductions in both grain yield and quality (Cakmak *et al.*, 1999; Rehman *et al.*, 2012; Hussain et al., 2012). Zinc deficiency in plants is common on alkaline and calcareous soils with pH> 8.0, and Zn availability in soils is inversely related to soil pH (Srinivasara *et al.*, 2008).

As Zn availability decreases in high-pH soils, Zn deficiency and B toxicity are simultaneously encountered in some soils of arid and semiarid regions (Singh *et al.*, 1990; Rajaie *et al.*, 2009; White and Zasoski 1999). Zinc deficiency may elevate B tissue concentration to a toxic level in barley (Graham *et al*. 1987) and wheat (*Triticum aestivum*) (Singh *et al.* 1990). Boron toxicity in Zn-deficient lemon (*Citrus limon*) seedlings could be alleviated by Zn application (Rajaie *et al.*, 2009). In contrast, Sinha *et al.* (2000) noted an increase in shoot B concentration in mustard (*Brassica campestris*) with increasing Zn application, suggesting complexity of the B x Zn interaction. Torun *et al.* (2001) has reported that Zn

application results in decreased shoot B concentration in 25 wheat cultivars, however, the response is quite variable in different cultivars.

Taking into account the simultaneous occurrence of B toxicity and Zn deficiency in some soils of arid and semiarid regions of Australia and the world, difficulties of ameliorating B toxicity in soil and thus desirability of genetic solutions, this study was designed to characterize the interaction between B and Zn supply using two barley genotypes that differ in tolerance to Zn deficiency and B toxicity.

MATERIALS AND METHODS

River sand used in the study was washed with deionised water and air-dried. It had pH 6.90, organic carbon 1 g kg^{-1}, P (Colwell) 2 mg kg^{-1}, DTPA-extractable Zn 0.15 mg kg^{-1} and hot-water-soluble B 0.1 mg kg^{-1}. One kg of sand was placed into plastic-bag-lined plastic pots. Basal nutrients (in mg kg^{-1} of dry sand) 221.4 KH_2PO_4, 139.9 K_2SO_4, 150.3 $CaCl_2.2H_2O$, 40.1 $MgSO_4.7H_2O$, 2 $CuSO_4.5H_2O$, 10 $MnSO_4.H_2O$, 0.2 $Na_2MoO_4.2H_2O$ and 95.2 NH_4NO_3, were applied in solution to the sand surface. The pots were arranged in completely randomized design with four concentrations of B (0.1, 0.5, 1.5 and 3.0 mg kg^{-1} of dry sand using boric acid) and two of Zn (0.1 and 1.0 mg kg^{-1} of dry sand using Zn sulphate) with three replicates.

Two barley genotypes, B-tolerant/Zn-efficient Algerian landrace Sahara 3771 (Sahara) and B-sensitive/Zn-inefficient Australian cultivar Clipper, were used in this study. Seeds were hand sorted to a uniform size, sterilized [by soaking in 70 % (v/v) ethanol for 1 minute, followed by 10% sodium hypochlorite for 30 seconds and were rinsed thrice with double-deionised water] and pre-germinated on filter papers in Petri Dishes. Five pre-germinated seeds were sown in each pot and were maintained to three plants per pot at two leaf stage. Pots were randomized and their positions were rotated within a block daily to minimize the effect of microenvironments.

Plants were grown in a glasshouse at the University of Western Australia, Perth (31□58' S, 115□49' E) from 12th February to 18th of March and were irrigated with double-deionized water daily keeping water content up to 90% field capacity (10% w/w). After 3 weeks, N @ 95.2 mg kg^{-1} sand was added in the form of NH_4NO_3.

Plants were harvested 35 days after sowing. Roots were rinsed under running deionised water, followed by rinsing in double-deionized water three times. Roots and shoots were then separated, and shoots were rinsed in double-deionised water followed by rinsing in 1% (v/v) acetic acid solution and again in double-deionised water to avoid any external Zn, attached to shoot.

Root and shoots were oven dried at 70 ºC for 72 hours, weighed, ground to pass a 40-mesh sieve, ashed at 550 ºC for 14 hours, solubilized in 10 mL of 30 % (v/v) hydrochloric acid (HCl) for 30 minutes at 50 ºC and analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Total Zn and B contents were calculated by multiplying shoot and root dry weights with shoot and root Zn and B concentration. Other calculations were done as follows:

$$\text{Relative yield} = \frac{\text{Dry matter at BH}}{\text{Dry matter at control}} \times 100$$

Control was 0.1 mg kg^{-1} B, and H refers to other B treatments.

$$\%\ \text{translocation} = \frac{\text{Zn or B content in shoots}}{\text{Total Zn or B content in whole plants}} \times 100$$

$$\text{Boron: Zinc (B:Zn) ratio} = \frac{\text{B concentration (m mol kg}^{-1})}{\text{Zn concentration (m mol kg}^{-1})}$$

The data were subjected to statistical analysis using Co-Stat software. Duncan's Multiple Range Test at $\alpha = 0.05$ was employed in pair-wise comparisons.

Table 1. Sum of squares of main and interactive effects (Zn fertilization, B addition and cultivars) on various growth and ionic parameters of barley.

Variables	Shoot dry matter	Root dry matter	Root:shoot ratio	Shoot B concentration	Root B concentration	Shoot Zn concentration	Root Zn Concentration
Zinc (Zn)	0.35**	0.47**	0.005 ns	602***	22 ns	517***	204**
Boron (B)	0.70***	7.135***	0.61***	37913***	7166***	119***	97***
Cultivars (C)	0.81***	2091***	0.92***	7864***	1808***	1339***	1216***
Interactions							
Zn x B	0.09 ns	0.019 ns	0.02*	328***	2.5 ns	4.32 ns	31.7***
Zn x C	0.09 ns	0.021 ns	0.015 ns	73 ns	0.10 ns	7.20 ns	20.8**
B x C	0.48***	1.38***	0.07***	1705***	255***	3.21***	0.88 ns
Zn x B x C	0.07 ns	0.03 ns	0.006 ns	89*	0.22 ns	10.25 ns	6.6 ns
Error	0.03	0.04	0.005	21	6.8	1.75	3.5

ns= non-significant, ** =significant at 0.01, *** = significant at 0.001

RESULTS

Plant biomass: There were significant main effects of B and Zn and cultivars (C) on shoot and root dry matter and root: shoot ratio (Table 1). The interactions among these factors were not significant, except for B x cultivar. Boron application up to 1.5 mg kg^{-1} did not affect shoot dry matter in either genotype. Nonetheless, compared with control (0.1 mg B kg^{-1}), a high dose of B (3.0 mg kg^{-1}) significantly ($p \leq 0.05$) reduced shoot dry matter in Clipper (50%), but not in Sahara. Overall, Clipper produced more shoot biomass than Sahara in all Zn and B treatments, except at 3.0 mg B kg^{-1} (Fig. 1A).

Genotypes differed significantly for root dry matter, with Sahara producing more root biomass than Clipper (Fig. 1B). Boron application above the control level significantly reduced root dry matter in both genotypes, overall reduction was more prominent in Clipper (60%) than Sahara (32%). Root dry matter was significantly greater in Sahara than Clipper at 1.5 and 3.0 mg B kg^{-1}, but there was no difference at the two lower B treatments, making the interaction cultivar x B, significant.

On the average in all treatments, root: shoot ratio, was significantly greater in Sahara than Clipper. Boron application reduced root: shoot ratio in both genotypes; this reduction was more prominent in Clipper than Sahara at higher B concentration (1.5 and 3.0 mg kg^{-1}).

Boron concentration in shoots and roots :There were significant B x Zn and B x cultivar interactions, regarding B concentration in shoots (Table 1). The variation between the cultivars ranged from 1.2-fold to more than 2-fold in different B treatments (Fig. 2A, B). Boron application significantly increased shoot B concentration in both cultivars, but the increase was more pronounced in Clipper than Sahara at both Zn levels.

Interestingly, high Zn application did not affect B concentration in shoots of both cultivars in treatments of up to 1.5 mg B kg^{-1} (Fig. 2A, B). However, Zn application (compared with 0.1 mg Zn kg^{-1} treatment) significantly decreased B concentration in Sahara (12%) and Clipper (19%) in the treatment with 3.0 mg B kg^{-1}.

There was a significant B x cultivar interaction with respect to root B concentration (Table 1). Application of B significantly increased B concentration in roots of both cultivars, but an increase was more pronounced in Clipper (ranging from 3- to 16-fold) than Sahara (1.8- to 14-fold) (Fig. 2C, D). Boron concentration in roots was significantly higher in Clipper than Sahara in all B treatments (except 0.1 mg kg^{-1}). Zinc application did not affect B concentration in roots regardless of the treatment.

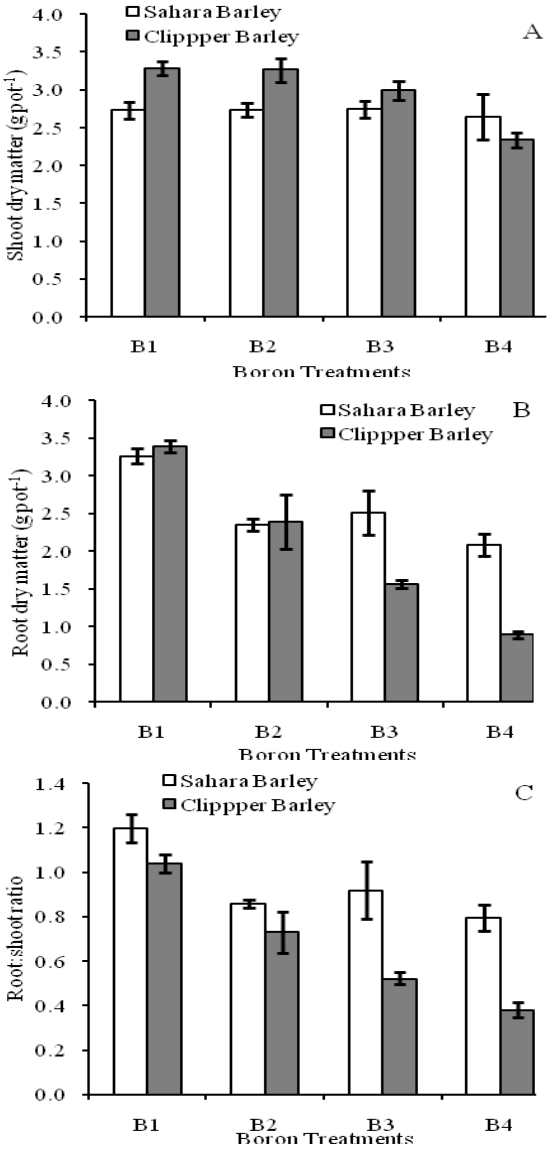

Figure 1. The interaction between B addition and cultivar treatments ($P<0.001$) influencing (A) shoot dry matter (LSD=0.28) (B) root dry matter (LSD=0.33) and (C) root:shoot ratio (LSD=0.04). The highest order interaction (Zn x B x cultivar) was not significant for the parameters shown. Values presented were averaged over the Zn treatment (0.1 and 1.0 mg Zn kg^{-1}) because it was not involved in any significant interaction (except Zn x B for root:shoot ratio, $P \leq 0.05$). Boron treatments were 0.1 (B1), 1.0 (B2), 1.5 (B3) and 3 mg B kg^{-1} soil (B4). The bars represent mean ±SE, n=6.

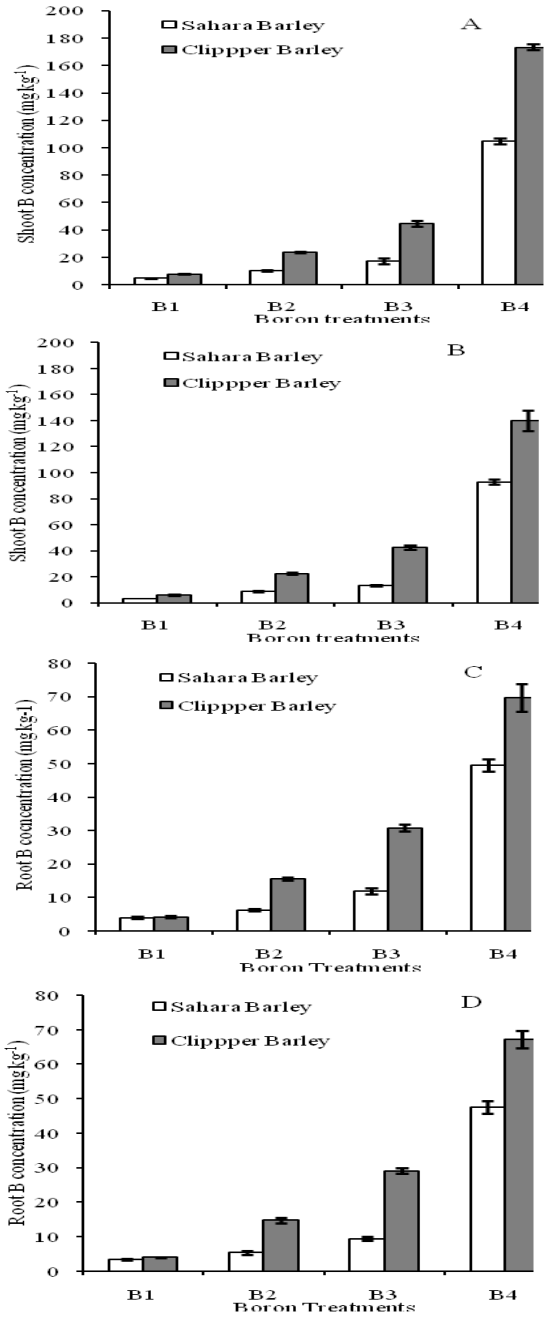

Figure 2. The interaction between B treatments and cultivar (P<0.001) influencing shoot B concentration at (A) 0.1 mg Zn kg^{-1} and (B) 1.0 mg Zn kg^{-1} (LSD=7.6) and root B concentrations at (C) 0.1 mg kg^{-1} Zn and (D) 1.0 mg kg^{-1} Zn (LSD=4.4). The highest order interaction (Zn x B x cultivar) was significant for shoot (p≤0.05) but not for root B concentration. Boron treatments were 0.1 (B1), 1.0 (B2), 1.5 (B3) and 3 mg B kg^{-1} soil (B4). The bars represent mean ±SE, n=3.

Boron contents in shoots and roots: There were significant main and interactive effects of Zn, B and cultivars for B contents in shoot and roots of barley plants (Table 1, Fig. 3). Clipper barley accumulated more B in shoots compared to Sahara. Application of Zn significantly reduced B contents in shoots of Sahara cultivar at low B levels, however at higher B levels, the differences were non-significant. Zinc induced reduction in B contents in shoots were more pronounced in Clipper at highest B level.

Contrarily, Zn application did not affect root B contents, however, differences between cultivars were significant at all levels of B and Zn. Clipper barley accumulated more B contents at both levels of Zn and all levels of B except at highest B level in soil.

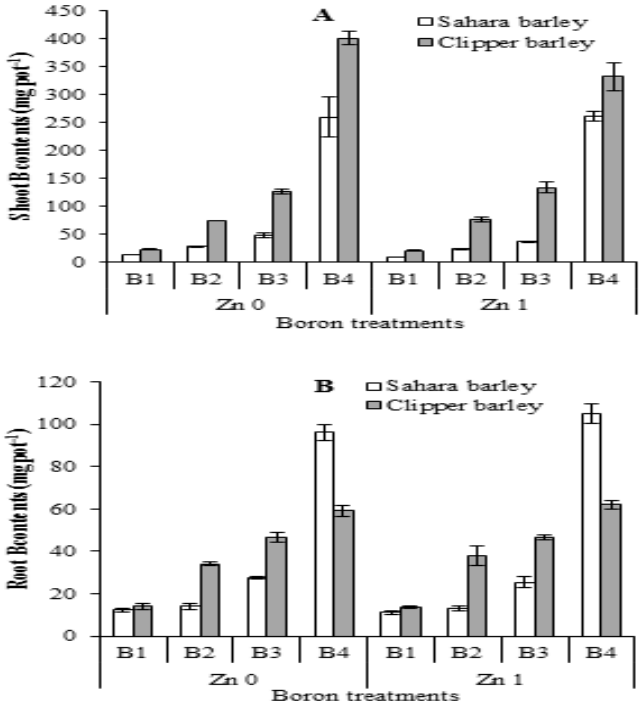

Figure 3. The interaction between B and Zn treatments and cultivar (P<0.001) influencing shoot B contents at (A) and root B contents (B). Zinc treatments were 0.1 and 1.0 mg Zn kg^{-1} soil while B treatments were 0.1 (B1), 1.0 (B2), 1.5 (B3) and 3 mg B kg^{-1} soil (B4). The bars represent mean ±SE, n=3.

Zinc concentration in shoots and roots : Shoot Zn concentration was significantly influenced by the B x cultivar interaction as well as the main effect of Zn (Table 1). Zinc concentration in shoots was significantly greater in Sahara than Clipper irrespective of B and Zn treatments (Fig. 4A & B). However, shoot Zn concentration in both cultivars decreased when B was applied at 3.0 mg kg^{-1} and this decrease was more in Clipper than Sahara.

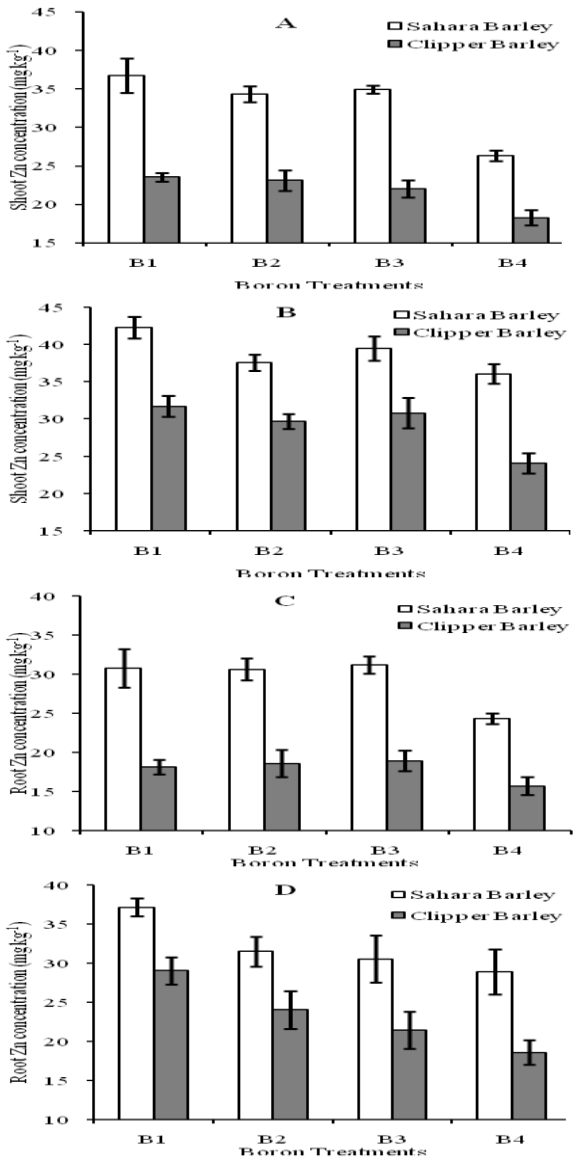

Figure 4. The interaction between B and Zn treatments and cultivar treatments (P<0.001) influencing shoot Zn concentration at (A) 0.1 mg Zn kg^{-1} and (B) 1.0 mg Zn kg^{-1} and root Zn concentrations at (C) 0.1 mg kg^{-1} Zn and (D) 1.0 mg kg^{-1} Zn. The highest order interaction (Zn x B x cultivars) was not significant for the parameters shown. Boron treatments were 0.1 (B1), 1.0 (B2), 1.5 (B3) and 3 mg B kg^{-1} soil (B4). The bars represent mean ±SE, n=3.

There were significant Zn x B and Zn x cultivar interactions, influencing Zn concentration in roots of barley (Table 1). Root Zn concentration was significantly (p<0.01) higher in Sahara than Clipper in all B and Zn treatments (Fig. 4C & D). Compared with control, the highest B application decreased root Zn concentration; however, at lower B rates (1.0 and 1.5 mg B kg^{-1}), Zn concentration was not affected.

Boron:zinc (B:Zn) concentration ratio in shoots and roots :There were significant main and interactive effects on B:Zn concentration ratio in shoots (Table 2). Similarly in roots, main and interactive effects of B levels, Zn levels and cultivars were highly significant on B:Zn concentration ratio except the highest order interaction (B x Zn x C). Zinc application decreased B:Zn ratio in shoots and roots of both cultivars, but the reduction was higher in plants grown with high B (3 mg B kg^{-1}) than in control plants (Fig 5).

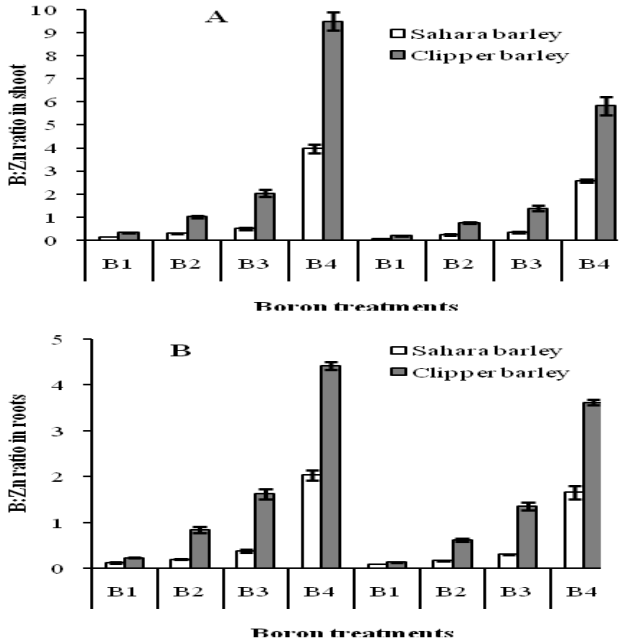

Figure 5. The interaction between B and Zn treatments and cultivars (P<0.01) influencing B:Zn (molar) ratio in shoot (A) and roots (B). All interactions were highly significant in both shoots and roots except highest order interaction in roots at P<0.01). Zinc treatments were 0.1 and 1.0 mg Zn kg^{-1} soil while B treatments were 0.1 (B1), 1.0 (B2), 1.5 (B3) and 3 mg B kg^{-1} soil (B4). The bars represent mean ±SE, n=3.

DISCUSSION

Boron toxicity results in a wide range of physiological effects, including decreased plant growth, and cell division and extension, and impaired photosynthesis and stomatal conductance (Gunes *et al*., 2000; Reid et al., 2004; Reid, 2010). The higher-B treatment (3.0 mg kg^{-1}) significantly reduced shoot biomass in Clipper barley (B-sensitive/Zn-inefficient), but had a non-significant effect in Sahara (B-

Table 2. Boron:zinc (concentration) ratio in shoots and roots of barley cultivars.

Zn treatment (mg kg^{-1})	B treatment (mg kg^{-1})	Shoot B:Zn concentration ratio		Root B:Zn concentration ratio	
		Sahara	Clipper	Sahara	Clipper
0.1	0.1	0.77±0.07	1.92±0.12	0.75±0.17	1.36±0.14
	0.5	1.73±0.42	6.05±0.19	1.20±0.12	4.98±0.70
	1.5	2.93±0.43	12.10±0.20	2.27±0.37	9.65±1.19
	3.0	23.50±0.10	56.20±0.10	12.00±1.10	26.10±0.89
1.0	0.1	0.46±0.11	1.11±0.11	0.53±0.01	0.82±0.09
	0.5	1.41±0.13	4.51±0.09	1.01±0.15	3.64±0.28
	1.5	2.01±0.04	8.21±0.0.04	1.82±0.10	8.06±0.82
	3.0	15.20±2.00	34.40±1.90	9.80±1.52	21.4±0.620
Mean SS	Zn	269***		24***	
	B	2535***		658***	
	Cultivars (C)	1095***		407***	
	Zn x B	144***		5.96***	
	Zn x C	62***		5***	
	B x C	381***		86**	
	Zn x B x C	27***		0.63 ns	

ns= non-significant, ** =significant at 0.01, *** = significant at 0.001

Table 3. Correlation coefficients (r) of dry matter accumulation or B tissue concentrations with root dry matter, and shoot and root B and Zn concentration, n = 48.

Parameter	Root dry matter	Shoot B conc.	Root B conc.	Shoot Zn conc.	Root Zn conc.
Shoot dry matter	0.50**	-0.56**	-0.53**	0.09 ns	0.06 ns
Root dry matter		-0.79**	-0.83**	0.57**	0.58**
Shoot B concentration			0.98**	-0.57**	-0.54**
Root B concentration				-0.57**	-0.54**

ns= non-significant, ** =significant at 0.01

tolerant/Zn-efficient) (Fig. 1; see also Hayes and Reid, 2004). In addition, visual B toxicity symptoms were observed only in the sensitive cultivar (Clipper), but not in Sahara.

Genotypes efficient in nutrient uptake or tolerant to ion stress generally allocate more photosynthates to roots, thereby increasing root: shoot ratio (Aziz et al., 2011). In the present study, increasing B application caused a more prominent reduction in root than shoot biomass in both cultivars (Fig. 1A, B), decreasing root: shoot ratio (particularly in Clipper; Fig. 1C). However, in contrast to shoot dry weight, root dry weight at 1.5 and 3.0 mg B kg^{-1} was significantly higher (in both absolute and relative terms) in Sahara than Clipper (Fig. 1B), suggesting that B tolerance is related to the maintenance of root growth under toxicity. Moreover, alteration in root morphology and variation in root distribution was observed in a range of species exposed to B toxicity (Choi et al., 2006).

Alleviation of B toxicity by Zn fertilization has been reported in many plant species (Graham et al., 1987; Singh et al., 1990; Gunes et al., 2000; Torun et al., 2001; Hosseini et al., 2007; Reid and Fitzpatrick, 2009). Application of Zn alleviated the reduction in plant dry matter (both shoots and roots) up to 1.5 mg B kg^{-1}, but not at the highest concentration of B (3.0 mg kg^{-1}). The impact of Zn application on improvement of root dry weight at high B was more prominent in Sahara (11%) than Clipper (6%).

Boron application significantly increased B concentration in shoots and roots of both cultivars, but this increase was higher in Clipper than Sahara (Fig. 2), probably due to loci on chromosomes 4H (*xWG114*) and 6H (*xAmy-1(a)*) associated with B exclusion and present in Sahara (Jefferies et al., 1999; Reid, 2007). Roessner et al. (2006) observed that huge variation in tissue tolerance among both Clipper and Sahara barley, but no biochemical mechanism could be identified. In contrast, it has been reported that under adequate to excessive supply, B is mainly taken up through passive influx (Dannel et al., 2002), with B-tolerant Sahara barley excluding B against the concentration gradient (Reid, 2010) via a B-efflux transporter (Hayes and Reid, 2004). In the study presented here, B-tolerant Sahara indeed had lower root and shoot concentrations than B-sensitive Clipper under high B supply, but B concentrations in shoots were greater than in roots (up to 2-fold in Sahara and 2.5-fold in Clipper at 3.0 mg B kg^{-1}) (Fig. 2). The physiological and molecular basis of B transport from roots to shoots remains to be elucidated.

Zinc application (1.0 mg Zn kg^{-1}) decreased shoot B concentrations in the highest B treatment (3.0 mg B kg^{-1}) in both cultivars. However, Zn application had a non-significant effect on root B concentration. It therefore appears that an alleviating effect of Zn in plants exposed to B toxicity might be related to decreased B transport from roots to shoots rather than decreased net B uptake from the external medium. A decrease in B concentration by Zn application has also been reported in shoots of maize (Hosseini et al., 2007) and wheat (Singh et al., 1990). Unfortunately, these reports did not contain any information on root B concentration. In Sahara cultivar, the B root concentration was maintained at a lower concentration in both Zn concentrations (Fig. 2), this result being expected. But it is also possible that B be actively extruded from the root in Sahara cultivar (Hayes and Reid, 2004) as it is clear from the lower B contents in roots of Sahara than in Clipper at all levels of B except at highest level (3.0 mg kg^{-1} B) (Fig. 3). Lower shoot and root B contents in Sahara indicated that this cultivar has both lower transport of B towards shoot as well as extrusion of B from roots. Recently Kumar et al. (2013) have reported two plasma membrane intrinsic proteins, OsPIP2;4 and OsPIP2;7 in rice, which are involved in mediating B transport and providing tolerance to boron toxicity through efflux from shoot and root tissues.

The B toxicity effects are generally correlated with the accumulation of B in shoots, which is a function of both the concentration of B in soil and the time of exposure (Reid, 2010). The significant correlations between root and shoot B concentrations, and root B concentration and shoot yield in the present study (Table 3) suggested that the main control over B toxicity is exerted at the root level. Tolerant cultivars of wheat and barley maintain relatively low B concentrations in roots (by effluxing B) and shoots (by restricting transfer of B from roots) (Reid, 2007).

There is no evidence that B toxicity is exerted through disruption of any process for which B is required (Reid, 2010). More likely, B toxicity arises from B affinity for some key metabolites and formation of strong complexes. Hence, B ratio with other nutrients (particularly Zn) may influence complexation of B and key metabolites. In the present study B:Zn concentration ratio in shoots and roots was higher in Clipper than Sahara (Fig. 3). This ratio correlated negatively with total dry matter (Fig. 4), suggesting the importance of maintaining a relatively low B:Zn ratio for tolerance to B toxicity.

Conclusion: Application of Zn alleviated B toxicity by moderating growth reduction and B accumulation, particularly, in B-sensitive cultivar Clipper. Lower root B concentration, lower translocations of absorbed B and higher Zn uptake resulting in lower B:Zn ratio in root and shoot tissues were associated with higher B tolerance in Sahara compared with Clipper.

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