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Influence of boron on seed germination and seedling growth of wheat (*Triticum aestivum* L.)

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A laboratory experiment was conducted at Department of Plant Science, Ambo University, Ethiopia, to study the effect of boron availability on seed germination and seedling growth of wheat (*Triticum aestivum* L. var *Danda'a*). Seeds were sown in petri dishes with varying concentrations of boron (0, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/L) at room temperature (24 ± 2°C) in complete randomized design (CRD) with four replications. Germination percentage and rate, shoot and root lengths, shoot and root fresh and dry weights, root number, root - shoot ratio and seedling vigor index were found to decrease beyond 0.25 mg/L, and phytotoxicity increased significantly (except on shoot at 0.25 mg/L) with increase in the concentration of boron in the germinating medium.

Key words: Boron, germination, phytotoxicity, seedling growth, tolerance, vigor, wheat.

INTRODUCTION

Boron (B) is a micronutrient required by plants in a very small quantity (Abd El-Wahab, 2008), and its availability in soil and irrigation water is an important determinant of agricultural production (Tanaka and Fujiwara, 2007). In soil solution, boron exists primarily as boric acid (H_3BO_3), which can be easily leached under high rainfall conditions (Yan et al., 2006) leading to plant deficiencies. On the contrary, under low rainfall conditions, boron cannot be sufficiently leached and therefore may accumulate to toxic levels for plant growth (Reid, 2007). This occurs very often in arid and semi arid regions where parent material and groundwater may contain high concentrations of boron. The accumulation of boron in top soil due to evaporation of groundwater reaches toxic levels that can reduce crop yields (Tanaka and Fujiwara, 2007). Boron is often found in high concentrations in association with saline soils and saline well water (Dhankhar and Dahiya, 1980). Of all the potential sources, irrigation water is the most important contributor to high levels of soil boron (Chauhan and Power, 1978). In assessing the potential toxicity of B rich irrigation water, the physical and chemical characteristics of the soil must be considered (Rauf et al., 2007). Boron can be regenerated through the mineralisation of soil organic matter, or through weathering processes of soil minerals (Peryea et al., 1985).

Plants exposed to excess of boron have reduced vigor, retarded development, leaf burn (chlorotic and necrotic patches in older leaves), and decreased number, size, and weight of fruits (Nable et al., 1997). Boron toxicity is an important agricultural problem that limits crop productivity in different regions of the world, and can occur in B-rich soils or in soils exposed to B-rich irrigation waters, fertilizers, sewage sludge, or fly ash (Luis et al., 2012).

Wheat (*Triticum aestivum* L.) is a staple food for more

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than 35% of the world population and is also the first grain crops in most of the developing countries (Metwali et al., 2011). Bread wheat is the main food of people in many countries and about 70% calories and 80% protein of human diet is supplied from its consumption (Taregh et al., 2011). In Ethiopia, the total area put to wheat production was estimated to be 1.68 million ha with an average yield of 1827 kg/ha (CSA, 2011). Abiotic stress, especially drought stress and boron toxicity, are worldwide problems which seriously constrain global crop production (Schnurbusch et al., 2010; Pan et al., 2002). It is one of the major causes of crop loss worldwide, which commonly reduces average yield of many crops by more than 50% (Wang et al., 2003; Bayoumi, 2008). In countries like Australia, B toxicity impacts heavily on wheat production (up to 11% yield reduction) in affected areas (Moody and Rathjen, 2007).

Shoot biomass reduction of wheat was observed owing to high B addition to soil (Wimmer et al., 2003). Wheat with high B concentrations showed leaf edge burning and necrosis compared with the control treatments (Sonmez et al., 2009). Genotypic variation in susceptibility to B toxicity has been reported (Torun et al., 2006). Paull et al., (1988) reported wide range of intra-specific variation in response to B occurs in a number of crops, including bread wheat (*Triticum aestivum* L). In addition, seed germination and seedling growth are the most important phases in the life cycle of plant, and are highly responsive to the existing environment. Hence, the current experiment was conducted to investigate the effect of boron on germination and seedling growth parameters of wheat (*Triticum aestivum* L. var *Danda'a*).

MATERIALS AND METHODS

A laboratory experiment was conducted in October, 2013 at the Department of Plant Sciences, Ambo University, to investigate the effect of boron on germination and seedling growth of wheat. The experiment was arranged in completely randomized design with four replications. Cultivar *Danda'a* was treated with eight levels of boron (0, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/L) for the experiment, deionized water was used for the control treatment. Boric acid (H_3BO_3) was used as a source of boron. Seeds were surface sterilized with 30% hydrogen peroxide solution for 5 min, and rinsed with deionized water. Twenty seeds were uniformly placed per Petri dish (9.5 cm diameter) using a forceps after the Petri dish were sterilized with 98% ethanol, and rinsed with deionized water. Filter papers were well soaked by adding 7 ml with the respective solutions (7 treatment solutions and the control) at an interval of 48 h as described by Naveed et al. (2001). All the Petri dishes were covered with lids and kept at room temperature (24±2°C). Germination continued for 10 days, and germinated seeds were counted daily. Germination was considered to have occurred when radicles attained a length of 2 mm. After 10 days, parameters such as percent germination and rate of germination were calculated according to ISTA (1999); and root and shoot lengths of seedling were measured using a scale. Root and shoot dry weights were recorded after oven drying for 72 h at 60°C. The seedling vigor index (SVI) was determined as Hosseein and Kasra (2011):

Seedling Vigor Index = % Germination × Seedling dry weight (g)

Tolerance index (T.I.) was determined by Iqbal and Rahmati (1992) method:

T.I. = (Mean root length in treatment solution / Mean root length in distilled water) × 100

The percentage of phytotoxicity on shoot and root of seedlings was calculated following the formula given by Chou and Lin (1976):

$$\text{Phytotoxicity of Shoot (\%)} = \frac{\text{Shoot length of control - Shoot length of treatment}}{\text{Shoot length of control}} \times 100$$

$$\text{Phytotoxicity of Root (\%)} = \frac{\text{Root length of control - Root length of treatment}}{\text{Root length of control}} \times 100$$

Relative water content (RWC) of seedling was calculated as per the formula used by (Shalaby et al., 1993):

$$\text{RWC} = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100$$

Statistical analysis of the data was performed using one-way ANOVA using SAS statistical software (Version 9). Based on the ANOVA results, mean separations were performed by Duncan's multiple range test at 5% level.

RESULTS AND DISCUSSION

Seed germination and seedling growth

The germination percentage at 0.25mg/l showed no difference compared to the control (Table 1). A significant decrease in germination was observed at boron concentrations higher than 0.5 mg/L. At 8 and 16 mg /L, wheat seed failed to germinate, indicating that germination is totally inhibited at such high concentra-tions of boron (Table 1). The consistent decrease in percentage and rate of seed germination beyond 0.25 mg/L in the present study is in line with the findings of Yau and Saxena (1997) and Muhammad et al., (2013) who stated that high boron concentration reduced germination percentage of durum wheat and maize, respectively.

The shoot and root lengths, shoot and root fresh and dry weights, and seedling dry weight decreased significantly with the increase in boron concentration (Tables 1 and 2). However, the highest shoot length (5.12 cm) and root length (5.31 cm) were obtained with 0.25 mg/L boron

Table 1. Effect of boron on germination, and shoot and root lengths of wheat.

Boron Conc. (mg/L)	Germination (%)	Germination rate	Shoot length (cm)	Root length (cm)
0	96.25[a]	5.39 (3.25)[a]	14.3 (4.78)[ab]	18.8(5.31)[a]
0.25	96.25[a]	4.97(3.15)[a]	17.1 (5.12)[a]	12.7(4.56)[b]
0.5	85[ab]	2.82 (2.68)[ab]	12.6(4.55)[b]	6.1 (3.46)[c]
1	77.5[bc]	2.25(2.5)[b]	7.4 (3.72)[c]	2.2(2.46)[d]
2	68.75[c]	1.64 (2.27)[bc]	3.4(2.73)[d]	1.4 (2.14)[de]
4	38.75[d]	0.8 (1.77)[c]	0.95(1.78)[e]	0.88(1.76)[e]
8	0[e]	0 (1.00)[d]	0(1.00)[f]	0(1.00)[f]
16	0[e]	0 (1.00)[d]	0(1.00)[f]	0(1.00)[f]
SEm (±)	10.4	0.39	0.36	0.35
CV (%)	18	17.5	11.7	12.9

Means with similar letters in each column are not significant at 5% level of probability. Data in parenthesis are square root transformed.

Table 2. Effect of boron on fresh and dry weights of wheat seedling.

Boron Conc. (mg/L)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Seedling fresh weight (g)	Seedling dry weight (g)
0	0.09 (1.306)[ab]	0.008(1.089)[a]	0.22(1.373)[a]	0.007(1.081)[a]	0.31(1.56)[a]	0.015(1.39)[ab]
0.25	0.11(1.332)[a]	0.011(1.106)[a]	0.06(1.240)[ab]	0.009(1.092)[a]	0.17(1.41)[ab]	0.020(1.14)[a]
0.5	0.07(1.260)[b]	0.009(1.092)[a]	0.03(1.177)[abc]	0.007(1.082)[a]	0.10(1.32)[bc]	0.015(1.12)[ab]
1	0.03(1.1 47)[c]	0.004(1.059)[b]	0.025(1.137)[bc]	0.006(1.073)[ab]	0.055(1.23)[cd]	0.009(1.10)[b]
2	0.009(1.097)[cd]	0.002(1.037)[c]	0.019(1.136)[bc]	0.003(1.049)[bc]	0.03(1.17) [cd]	0.004(1.06)[c]
4	0.006(1.065)[d]	0.002(1.029)[c]	0.014(1.095)[bc]	0.003(1.040)[c]	0.02(1.14)[de]	0.004(1.06)[c]
8	0(1.0)[e]	0(1.0)[d]	0(1.0)[c]	0(1.0)[d]	0(1.0)[e]	0(1.0)[d]
16	0(1.0)[e]	0(1.0)[d]	0(1.0)[c]	0(1.0)[d]	0(1.0)[e]	0(1.0)[d]
SEm(±)	0.03	0.014	0.13	0.017	0.107	0.018
CV (%)	3	1.3	11.2	1.6	8.8	1.7

Means with similar letters in each column are not significant at 5% level of probability. Data in parenthesis are square root transformed.

concentration and control, respectively; and the lowest shoot and root lengths were with 8 mg/L and 16 g/L concentrations that caused a complete failure of germination. Boron inhibits root growth primarily through limiting cell elongation rather than cell division (Brown et al., 2002). Nable et al. (1997) also reported that shoot and root growth reduced when exposed to high B levels.

Shoot and root fresh and dry weights, and seedling fresh and dry weights decreased significantly with increase in boron concentration as compared to control and 0.25 mg/L (Table 2). Fresh weight and dry matter yield of the plants decreased significantly with increasing levels of applied boron (Ayvaz et al., 2012; Alpaslan and Gunes, 2001). Muhammad et al. (2013) and Turan et al. (2006) reported that shoot and root fresh and dry weights of maize and wheat decreased with the increase in the concentration of boron, respectively. Boron at 0.25 mg/L concentration resulted in the highest shoot fresh weight (1.33 g), shoot (1.1 g) and root (1.09 g) dry weights, and seedling dry weight (1.14 g). The significant increase at low concentration of boron could be due to its involvement in cell elongation or cell division and meristematic growth (Khan et al., 2006). Bonilla et al. (2004) and Farr (2010) reported that low concentrations of exogenous boric acid stimulated seed germination and seedling growth, while high concentrations showed an inhibitive effect on these parameters (Ölçer and Kocaçalışkan, 2007). Metwally et al. (2012) reported a gradual reduction in fresh and dry matter yield of shoots and roots, with increasing boron concentration in sand culture media.

Root number and root- to-shoot ratio

Root number and root-to-shoot ratio showed no significant difference up to 0.5 and 0.25 mg/L of boron concentrations, respectively. Further increase in boron concentrations significantly reduced both root and root-to shoot ratio (Figure 1). Cokkizgin (2013) reported similar

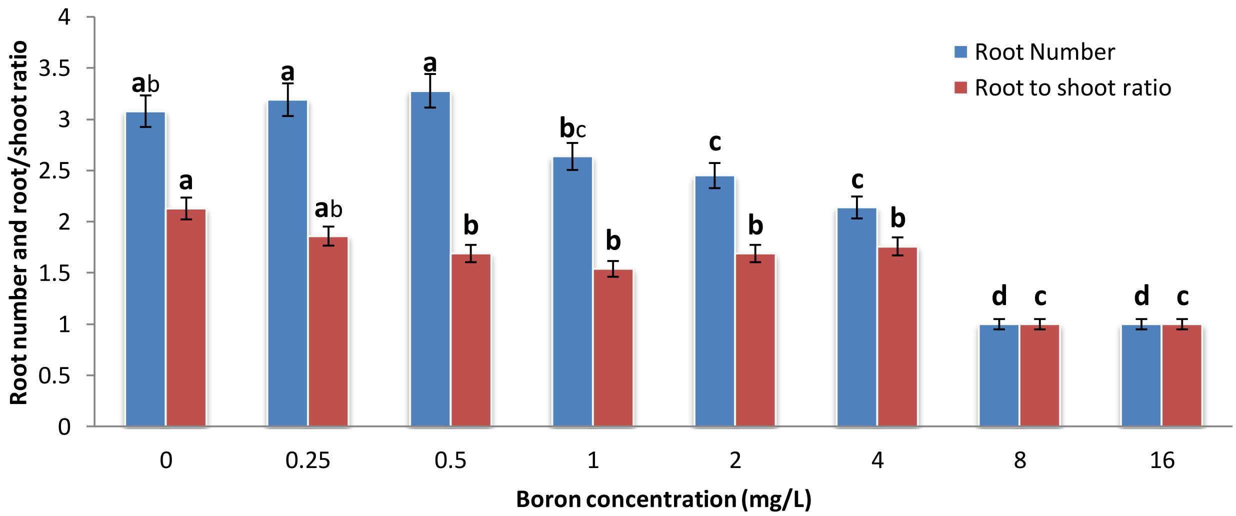

Figure 1. Effect of boron on root number and root /shoot ratio in wheat.

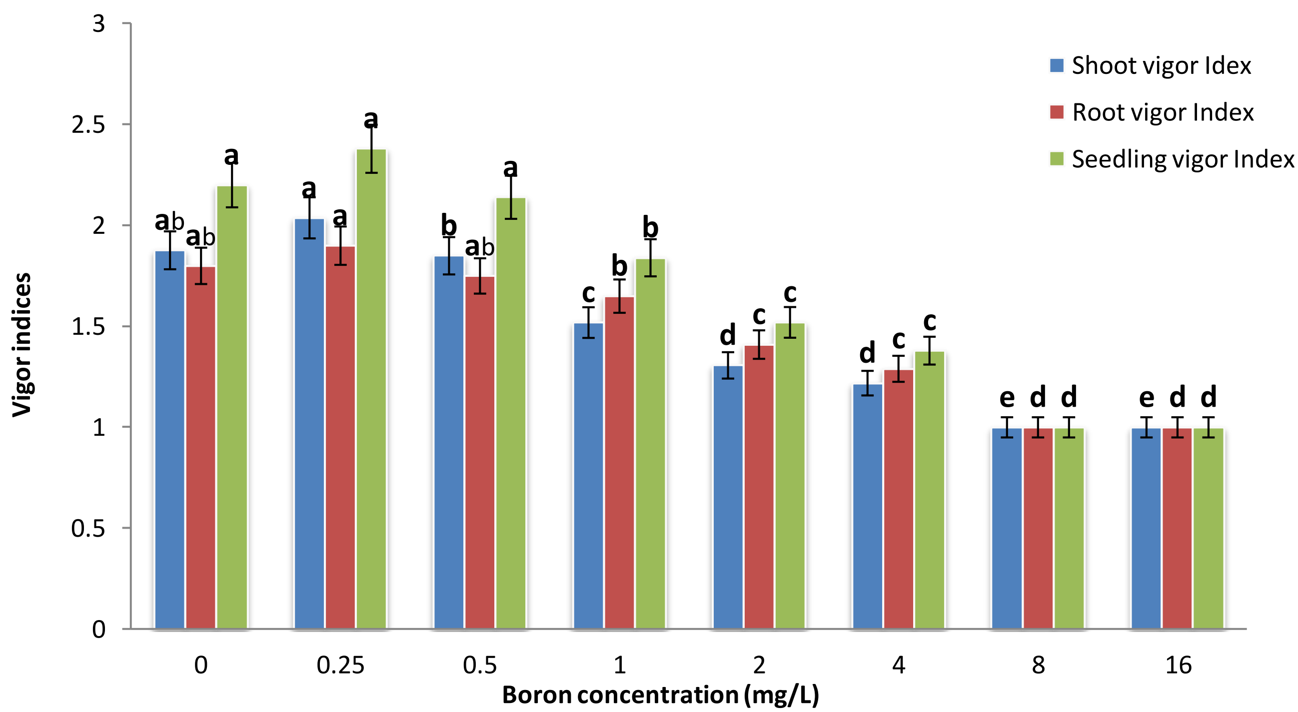

Figure 2. Effect of boron on vigor indices of Wheat.

findings on bean, and the increase in B concentration inhibited the secondary root emergence (Rehman et al., 2012).

Seedling vigour, tolerance and phytotoxicity

Boron concentrations showed a significant effect on seedling vigour index, shoot and root vigour indices, and tolerance index (Figure 2). The highest value for each trait was noticed in control treatment and 0.25 mg/L. Boron concentrations above 0.5 mg/L decreased vigour indices and tolerance index. Similar findings were reported by Mirshekari (2012) and Cokkizgin (2013), who observed a restricted, seedling vigor index of dill and

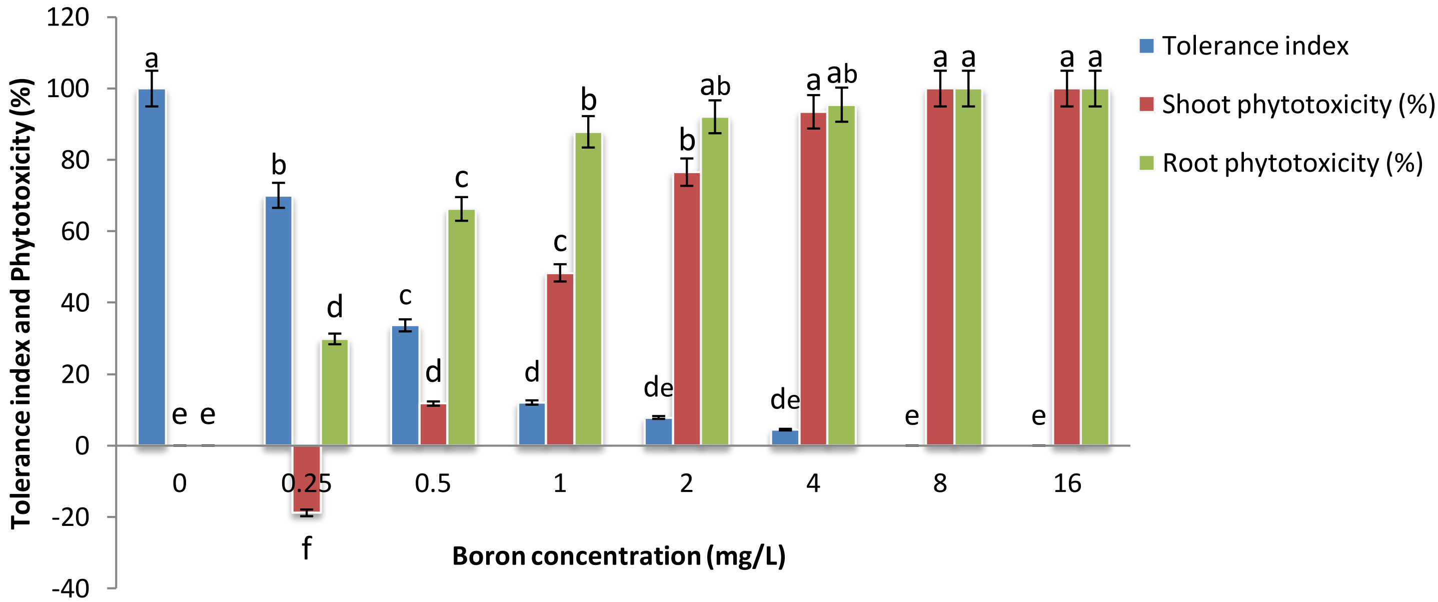

Figure 3. Effect of boron on tolerance index and phytotoxicity of wheat.

Phaseolus vulgaris at high level of boron concentrations, respectively. Ivanova et al., (2010) also reported a decrease in rapeseed seedling vigor indices with increasing micronutrient concentrations.

Boron showed a significant effect (p<0.05) on shoot and root phytotoxicity (Figure 3). Phytotoxicity of shoot and root increased with the increase in boron concentration. Lesser shoot and root toxicity was recorded with control treatment and lower concentration (0.25 mg/L), while it increased at higher concentration. At 0.25 mg/L B concentration shoot growth was highly promoted compared to control, hence showed a negative toxicity. Maximum phytotoxicity of boron on shoot and root was observed with ≥8 mg/L. The finding of our study is in agreement with the recent reports of Shaikh et al. (2013) and Habtamu et al. (2013) who reported that micronutrient toxicity of shoot and root decreased at lower concentration. On the other hand, it has the characteristics of promoting seedling growth at low concentrations based on crop types and varieties; however, its toxicity increased with increased in concentrations.

The tolerance index of wheat seed declined significantly with the increase in boron concentration. The maximum value of the tolerance index was obtained in the control treatment (100%) followed by 0.25 mg/L (70.1%), while a zero tolerance was attained for boron concentrations ≥8 mg/L. This result is in agreement with the recent findings of Shaikh et al. (2013) and Habtamu et al. (2013) who reported that increasing micronutrient concentrations decreased wheat and tomato tolerance index, respectively.

Relative water content

Increased boron concentrations ≥8 mg/L caused significant decrease in shoot and root relative water content of seedlings (Figure 4). In boron concentrations between 0 and 4 mg/L, the relative shoot and root water content decreased from 91.4 to 60.6%, and 85.8 to 61.4%, respectively. The highest values of relative water content of shoot and root were observed in the control, while the lowest in boron concentrations higher than ≥4 mg/L. High water content on shoot and root implies the higher dilution effect of boron that reduces its toxicity. This result was in agreement with Kinfemichael (2011) who found a drastic decrease seedling shoot and root relative water content at higher salinity level.

Conclusion

High boron concentrations caused a decrease in germination and germination rate, shoot and root lengths, shoot and root fresh and dry weights, vigor, tolerance and toxicity indices and relative water content of shoot and root in wheat. Low boron concentration (0.25 mg/L) showed the highest shoot fresh weight, shoot and root dry weights, seedling dry weight, and the lower shoot toxicity index. At higher boron concentrations, a deleterious effect on germination and seedling growth traits of wheat (*Triticum aestivum* L. var. *Danda'a*) was observed.

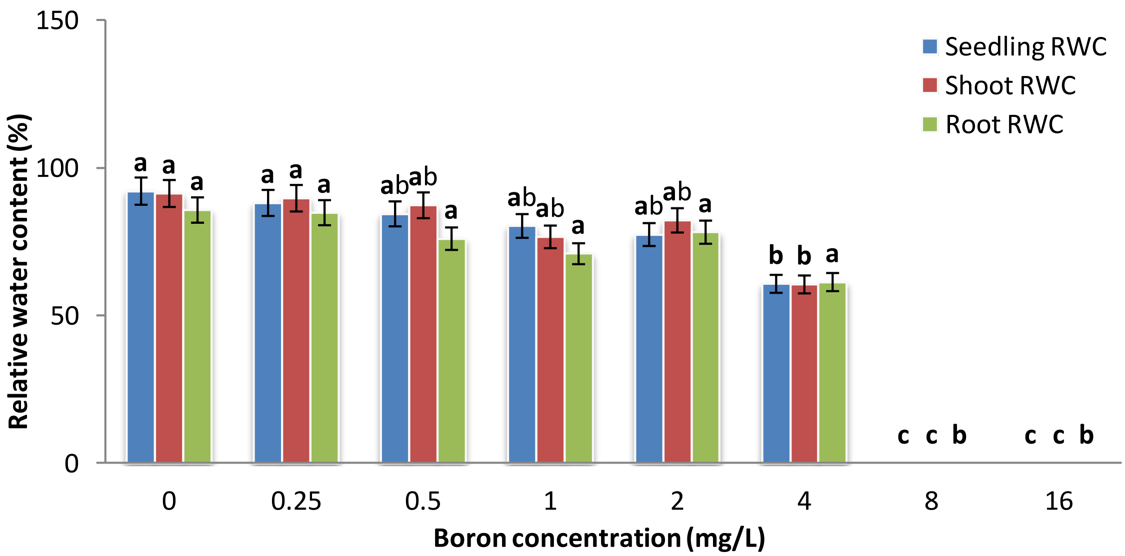

Figure 4. Effect of boron on shoot and root relative water content of wheat.

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