

Effect of elevated boron concentrations on the growth and yield of barley (*Hordeum vulgare* L.) and alleviation of its toxicity using different plant growth modulators**Soad Soliman El-Feky^{1*}, Fatma Aly El-Shintinawy^{1,2}, Eman Mohamed Shaker³ and Hassan Aly Shams El-Din³**¹Botany Department, Faculty of Science, Tanta University, Tanta, Egypt²Present address: Biology Department, Faculty of Science, Taif University, Taif, Kingdom Saudi Arabia³Soil, Water and Environment Research Institute, Sakha Agricultural Research Station, Sakha, Egypt

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Abstract

This study was carried out to assess the role of elevated concentrations of boron in barley (*Hordeum vulgare* L. cv. Giza 123) growth and yield as well as to test the involvement of some plant growth modulators in counteracting the boron-mediated retardations to improve its tolerance. Barley grains were treated with B concentrations of 0, 0.5, 1.5, 3.0 and 6.0 mg L⁻¹ (as boric acid). The alleviation of the growth inhibition in the presence of 3.0 mg L⁻¹ B was investigated by adding the following growth modulators: glutamic acid (1 or 3 mM), glycine (1 or 3 mM), ascorbic acid (2 or 5 mM), salicylic acid (1 or 5 mM) and calcium chloride (1 or 5 mM). Barley grains were cultivated and samples were collected at vegetative (22-day-old), flowering (45-day-old) and harvesting stages (92-day-old). At vegetative stage, the application of 0.5 mg L⁻¹ boron significantly increased ($P \leq 0.05$) shoot fresh weight by 1%. The addition of 0.5 and 1.5 mg L⁻¹ boron significantly increased ($P \leq 0.05$ and 0.01) growth parameters (shoot and root fresh and dry weights) by 5%, leaf area by 4.5% and 7%, Chl a and Chl b contents by 3% and 7% at vegetative and flowering stages, respectively, and yield by 5.5%, compared to non-boron treated barley. The inhibitory effects of boron on barley growth started at concentrations above 3.0 mg L⁻¹, causing decrease in all the measured parameters. The five tested growth modulators alleviated boron toxicity at 3.0 mg L⁻¹ at the following sequence: salicylic acid (1 mM), calcium chloride 5 mM \geq , ascorbic acid (2 mM) \geq , glycine (1 mM) and glutamic acid (3 mM). The boron-alleviating efficiency of either 1 mM salicylic acid or 5 mM calcium chloride recommended their application when cultivating barley in boron contaminated soils.

Keywords: Ascorbic acid; calcium chloride; growth criteria; salicylic acid.**Abbreviations:** AA- ascorbic acid; GA- glutamic acid; Gly- glycine; NB-non-boron; SA- salicylic acid.**Introduction**

Boron (B) is an essential nutrient for normal growth of higher plants and its availability in soil and irrigation water is an important determinant of agricultural production (Saleem et al., 2011). Boron deficiency causes different effects on very diverse processes in vascular plants such as root elongation, indoleacetic acid oxidase activity, sugar translocation, carbohydrate metabolism, nucleic acid synthesis, and pollen tube growth (Blevins and Lukaszewski, 1998; Goldbach and Wimmer, 2007; Saleem et al., 2011).

Besides, B can be involved in many processes as membrane potential, plasmalemma-bound enzymes and ion fluxes across membranes (Blaser-Grill et al., 1989; Goldbach et al., 2001), cytoskeletal proteins (Yu et al., 2003), accumulation of phenolics and polyamines and nitrogen metabolism (Camacho-Cristobal et al., 2004; 2005; Camacho-Cristobal and Gonzalez-Fontes, 2007). For many plant species there is only a narrow range in critical tissue concentrations between boron deficiency and boron toxicity (Blamey et al., 1997). Boron toxicity exerts different effects on vascular plants, such as reduced root cell division, lower photosynthetic rates, and decreased lignin and suberin levels (Nable et al., 1997; Reid, 2007). Accordingly, a reduced growth of shoots and roots is typical of plants exposed to high B levels (Nable et al., 1990). Different plant species respond differently to different levels of boron. B-deficiency

reduced photosynthetic efficiency of sunflower leaves (El-Shintinawy, 1999). Boron application increased the rate of fruiting of grain crops and decreased the incidence of bare ears in maize and decreased empty pods of soybean and empty grains in rice (Li and Liang, 1997). Effects of foliar applications of B to soybean included increased yield and larger seed size (Gascho and McPherson, 1997). Although the emergence of pea was affected by excess boron, the plant height and the number of nodes were reduced (Bagheri et al., 1992).

Besides, yield reduction by 10 to 20% was estimated in rice tolerant varieties irrigated with the excess boron during rainless dry seasons (Koohkan et al., 2008). Ayfer et al. (2006) reported that the total amount of boron in wheat shoots showed a highly significant negative correlation with decreases in shoot dry weight under boron supply. In tomato plants, excess boron depressed fresh and dry weights and reduced the yield (Eraslan et al., 2007a; Kaya et al., 2009). Fresh and dry plant matter of melon plants decreased significantly with increasing B concentrations, while B concentration of plant leaves, stems, and fruits increased significantly with increasing B (Goldberg et al., 2003). Tariq and Mott (2006) observed that toxic effects accompanied by considerable yield decreases at higher levels of B supply in radish. In barley, the symptoms of boron toxicity developed

on mature foliage, usually starting as chlorotic leaf tips or chlorotic patches at leaf margins near the leaf base and eventually turned necrotic and leaves sometimes dropped (Kluge and Podleska, 1985; Poole et al., 1991). Addition of boron to the soil increased the symptoms of boron toxicity in barley. These symptoms include decreased shoot growth and substantial reduction in grain and straw yield (Riley 1987, Moody et al., 1993; Nable et al., 1997).

The role of growth modulators in alleviation of boron and other abiotic stresses is clearly evident in many plants. Wassif et al. (1999) reported that the yield of sugar beet plants significantly increased by increasing sulfur level at any levels of excessive boron. In addition, Gunes et al. (2000) indicated that Zn treatments reduced the inhibitory effect of boron on growth of tomato plants. Gunes et al. (2007a) concluded that silicon alleviates the sodicity and B toxicity in spinach and tomato plants grown in boron toxic soil. Amer and Katta (1990) stated that the exogenous addition of certain amino acids such as proline, glutamic acid and arginine at seedling stage in barley minimized the loss of relative grain yield under salinity condition through increasing the plants salt tolerance capacity. The ability of salicylic acid (SA) to produce a protective effect on plants under external stress conditions has raised a considerable interest in researchers. Mishra and Choudhuri (1999) found that deterioration of heavy metal stresses was partially alleviated by exogenous application of salicylic acid in *Oryza sativa*. The exogenous application of 0.5 mM of soil incorporated salicylic acid significantly decreased the hazardous effect of boron toxicity in maize (Gunes et al., 2007b). Salicylic acid application positively affected the dry weight storage root of carrots grown under boron toxicity (Eraslan et al., 2007b). In plants, calcium ions are ubiquitous signaling second messengers. There are a number of external stimuli lead to changes in cytosolic calcium concentrations, which in turn, regulate a wide variety of responses and several physiological processes (Bush, 1995). Nable et al. (1997) showed that addition of Ca in irrigation water may result in reduction in B accumulation in plants. Sotiropoulos et al. (1999) reported that Ca partially protected kiwifruit plants against the harmful effects of B excess. The degree of tolerance was increased by increment of Ca concentration, where high Ca concentration reduced the boron absorption. Also, the high B and Ca levels in the nutrient solution decreased P, K, Mg, and Zn concentrations. Turan et al. (2009) concluded that dry shoot and root of wheat plants were strongly depressed and decreased by boron; however, Ca applications reduced the inhibitory effect of B on the plant growth.

Boron toxicity is a serious economic problem for agriculture in dry areas of West Asia and North Africa including Turkey, Syria, Iraq, Jordan, Egypt, Libya and Morocco (Yau and Ryan, 1994). High B concentration may occur naturally in soil and ground waters, or be added to the soil from mining, fertilizers, irrigation water, flying ash and industrial pollution (Nable et al., 1997; Alpaslan and Gunes, 2001).

Moreover, the poor drainage of saline soils may be responsible for excessive accumulation of boron in the soil solution (Grieve and Poss, 2000). Re-use of drainage water is a necessary alternative in the Northern part of Nile Delta region in Egypt due to the shortage of fresh water. Therefore, farmers use irrigation water from open drains, which has boron content derived from sewage and/or industrial effluents (Abo-Waly et al., 1997).

In our knowledge, little is known about the involvement of growth modulators such as salicylic acid, calcium chloride, ascorbic acid, and the amino acids glycine and glutamic acid in counteracting with the B-mediated retardations in barley. Therefore, this study was carried out to understand the role of B deficiency and toxicity in barley growth and yield as well as to test the involvement of some plant growth modulators.

Results

Growth parameters

Application of 0.5 and 1.5 mg L⁻¹ boron increased shoot and root fresh weights of barley at vegetative and flowering stages, compared to control [non-boron (NB) treated plants] (Table 1). The maximum increase in shoot and root weights were obtained with application of 1.5 mg L⁻¹ B. However, increment of B concentration up to 6.0 mg L⁻¹ decreased both parameters at both growth stages.

The maximum decrease for shoot and root fresh weights was observed in application of 6.0 mg L⁻¹ B. Two-way ANOVA revealed highly significant effect of different boron concentrations ($P \leq 0.05$ and 0.01) on shoot and root fresh weights of barley, compared to NB control plants at both vegetative and flowering stages. Application of 0.5 and 1.5 mg L⁻¹ B increased shoot and root dry weights of barley during all measured growth stages (Table 1). Gradual increase in B concentration up to 6.0 mg L⁻¹ had a reverse effect on both parameters. Application of 1.5 mg L⁻¹ B increased shoot height about 1% and 5% and leaf area by about 4.5% and 7% at vegetative and flowering stages, respectively, as compared to control. However, addition of 3 and 6 mg L⁻¹ B significantly decreased shoot height about 18% and 28% at vegetative stage and about 31% and 49% at flowering stage, respectively. Leaf area was significantly decreased with application of 3 and 6 by 20% and 30% at vegetative and by 32% and 50% at flowering stage, respectively (Table 1).

Chl a and Chl b content

At vegetative stage, increasing B concentration up to 1.5 mg L⁻¹ B increased Chl a and Chl b, compared to control (Table 2). The Presence of both 3 and 6 mg L⁻¹ B in the nutrient solution caused a highly significant decrease of both Chl a and Chl b. At flowering stage, increasing B concentration to 1.5 mg L⁻¹ B increased Chl a and Chl b compared to those of control (Table 2). Presence of both 3.0 and 6.0 mg L⁻¹ B in the nutrient solution caused a highly significant decrease of both Chl a and Chl b.

Yield parameters

Addition of 0.5 mg L⁻¹ B significantly increased the weight of straw and grains by 5.5%, as compared control. Increasing B concentration to 1.5 mg L⁻¹ caused a significant increase in straw and grain weight (Table 3).

On the other hand, straw and grains weights showed a highly significant decrease in response to application of 3 and 6 mg L⁻¹ B, compared to control. Weight of 100-grains was increased by about 3% and 7% by the addition of 0.5 and 1.5 mg L⁻¹ B, while application of 3 and 6 mg L⁻¹ B decreased this parameter by about 24% and 31% compared to those of

Table 1. Shoot and root fresh and dry weights (g plant⁻¹) of barley treated with different boron concentrations at vegetative and flowering stages.

Stage	Boron concentration (mg L ⁻¹)	Shoot fresh weight (g plant ⁻¹)	Root fresh weight (g plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)	Shoot height (cm)
Vegetative	0.0	2.48 ± 0.25	0.22 ± 0.003	0.400 ± 0.00	0.040 ± 0.000	21.7 ± 2.0
	0.5	2.71 ± 0.33 *	0.23 ± 0.004 ns	0.405 ± 0.06 ns	0.040 ± 0.002 ns	22.0 ± 1.3 ns
	1.5	2.75 ± 0.13 *	0.24 ± 0.003 *	0.411 ± 0.02 *	0.041 ± 0.001ns	22.2 ± 1.3 ns
	3.0	2.24 ± 0.20 *	0.16 ± 0.003 **	0.335 ± 0.10**	0.030 ± 0.001 **	17.8 ± 1.4**
	6.0	1.86 ± 0.67 **	0.13 ± 0.004 **	0.278 ± 0.02 **	0.020 ± 0.001 **	15.0 ± 1.2**
	L.S.D 0.05	0.21	0.02	0.01	0.01	2.30
	L.S.D 0.01	0.29	0.03	0.01	0.01	3.27
Flowering	0.0	4.14 ± 0.21	0.66 ± 0.02	0.57 ± 0.004	0.110 ± 0.000	73.8 ± 1.02
	0.5	4.29 ± 0.28 **	0.69 ± 0.03 *	0.58 ± 0.006 *	0.111 ± 0.001 *	76.5 ± 0.63 *
	1.5	4.33 ± 0.24 **	0.71 ± 0.03 **	0.59 ± 0.014 **	0.113 ± 0.001**	77.8 ± 1.97 **
	3.0	3.08 ± 0.22 **	0.48 ± 0.04 **	0.42 ± 0.003 **	0.080 ± 0.001 **	53.0 ± 1.70 **
	6.0	2.28 ± 0.28 **	0.39 ± 0.03 **	0.31 ± 0.014 **	0.060 ± 0.002 **	37.5 ± 1.10 **
	L.S.D 0.05	0.09	0.01	0.01	0.001	2.64
	L.S.D 0.01	0.13	0.02	0.01	0.002	3.84

* Results significantly different from control at (P< 0.05). ** Results significantly different from control at (P< 0.01). ns: non significant.

Table 2. Leaf area and Chlorophyll (a and b) contents (mg/cm²) of barley treated with different boron concentrations at vegetative and flowering stages.

Stage	Boron concentration (mg L ⁻¹)	Leaf area (cm ²)	Chl a (mg/cm ²)	Chl b (mg/cm ²)
Vegetative	0.0	4.4 ± 0.02	46.8 ± 0.95	22.5 ± 0.38
	0.5	4.5 ± 0.03 ns	47.6 ± 1.0 ns	23.4 ± 0.46 ns
	1.5	4.6 ± 0.07 *	48.2 ± 2.0 ns	24.1 ± 0.67 *
	3.0	3.5 ± 0.03 **	40.0 ± 2.1 **	19.5 ± 0.57 **
	6.0	3.0 ± 0.12 **	33.2 ± 1.3 **	17.8 ± 0.83 **
	L.S.D 0.05	0.114	2.76	1.47
	L.S.D 0.01	0.210	3.92	2.51
Flowering	0.0	23.3 ± 0.5	92.6 ± 1.2	44.3 ± 1.0
	0.5	24.5 ± 0.3 *	93.6 ± 1.1 *	45.1 ± 1.6 *
	1.5	24.9 ± 0.5 **	94.5 ± 1.3 **	45.9 ± 1.4 **
	3.0	16.4 ± 1.0 **	80.0 ± 2.0 *	37.0 ± 2.0 **
	6.0	11.7 ± 0.7 **	70.9 ± 1.7 **	33.4 ± 2.2**
	L.S.D 0.05	1.1	0.96	0.63
	L.S.D 0.01	1.6	1.02	0.98

* Results significantly different from control at (P< 0.05). ** Results significantly different from control at (P< 0.01). ns: non significant.

Table 3. Weight of straw and grains (g/20 individuals), weight of 100-grains (g) and B content (mg/kg DW) of barley grains treated with different B concentrations.

Boron concentration (mg L ⁻¹)	Straw	Grains	Weight of 100-grains	B
0.0	18.0 ± 1.0	10.8 ± 0.9	2.9 ± 0.25	0.32 ± 0.02
0.5	19.0 ± 1.2*	11.4 ± 1.3*	3.0 ± 0.30 ns	0.38 ± 0.02*
1.5	19.7 ± 1.4*	11.9 ± 1.0*	3.1 ± 0.21*	0.41 ± 0.02*
3.0	11.0 ± 2.0**	6.6 ± 1.1**	2.2 ± 0.32**	0.46 ± 0.03*
6.0	7.8 ± 2.3**	4.7 ± 1.8**	2.0 ± 0.34*	0.51 ± 0.03*
L.S.D 0.05	0.98	0.58	0.11	0.05
L.S.D 0.01	1.91	1.14	0.21	0.10

* Results significantly different from control at (P< 0.05). ** Results significantly different from control at (P< 0.01). ns: non significant.

control, respectively. Besides, the addition of 6.0 mg L⁻¹ B increased boron content in grains by about 59%.

Alleviation of boron toxicity

The highest efficiency in alleviation of adverse effects of B was obtained in salicylic acid (SA) and CaCl₂ among the five applied modulators, which followed by AA, Gly and GA, respectively (Table 4). The maximum significant recovery of shoot fresh weight of barley, treated with 3.0 mg L⁻¹ B was induced by 18% under 1 mM SA application at vegetative stage. However, at flowering stage the highest recovery (34%) was obtained by 5 mM CaCl₂ applications. On the

other hand, the maximum recovery of root fresh weight of barley treated with 3 mg L⁻¹ B was induced under 1 mM SA and 5 mM CaCl₂ application at vegetative (25%) and flowering (35%) stages, respectively. Based on these observations, mineral contents were determined at 3 mg L⁻¹ B treated barley under addition of 5 mM CaCl₂ and 1 mM SA (Table 5).

The presence of 1 mM SA or 5 mM CaCl₂ in the nutrient solution induced a reduction in B content in tissues of B-treated plants during all growth stages (the maximum decrease of 36% for shoots and 32% for roots was observed). In addition, the presence of 1 mM SA or 5 mM CaCl₂ in the nutrient solution reduced the P content in B-treated barley

tissues during all growth stages (the maximum decrease was 41% for shoots and 43% for roots). Also, the presence of 1 mM SA or 5 mM CaCl₂ in the nutrient solution reduced K content in B-treated barley tissues during all growth stages (the maximum decrease was 41% for shoots and 40% for roots). Moreover, the presence of 1 mM SA acid or 5 mM CaCl₂ in the nutrient solution reduced N content in B-treated barley tissues during all growth stages (the maximum decrease of was observed for both shoots and roots).

Discussion

The present study showed that barley growth was affected differently at various B levels (Table 1). When B was applied as 0.5 and 1.5 mg L⁻¹, all of the measured growth parameters significantly increased, especially during the flowering stage as compared to those of control.

Greater leaf area coupled with higher Chl a and Chl b contents were also detected at the same B concentrations (Table 2), suggesting that sufficient B concentration can promote barley leaf development. Moreover, straw and grains weights, yield parameters and B accumulation in barley grains were significantly increased under both B concentrations. The results were in agreement with the findings of Hu and Brown (1997) and Takano et al. (2005). Yu and Bell (1998) indicated that addition of boron in the nutrient solution increased dry matter and plant height of rice. Shoot and root mass of tobacco plants treated with only low B concentration was decreased, compared to plants sufficiently supplied with B (Hu and Brown, 1994).

In intact plant tissue the boron exists in water-soluble forms. The majority of boron seems to be localized in the apoplasmic region as boric acid and water insoluble forms under stress condition (Mahboobi et al., 2001). This might be the main cause of tolerance induced under sufficient (optimum) boron application. The vital roles of boron are related to its capacity to form diester bridges between *cis* hydroxyl-containing molecules such as those present in plant cell walls and membranes (Bolanos et al., 2004; Nuttall, 2000).

Rapid boron-induced changes in membrane function could be attributed to the formation of boron complexing membrane constituents. The results showed that the addition of 3 mg L⁻¹ boron has its inhibitory effects on barley growth. Boron toxicity resulted in a decrease in all the measured growth parameters. Lesser leaf area coupled with decreased Chl a and Chl b contents were also detected at the same B concentration, which consequently declined leaf development. Moreover, yield parameters were also decreased while B concentration in grains was significantly increased. These inhibitions reflected the toxic effect of excessive B in the nutrient solution on barley growth and yield. These results are in agreement with those obtained by Gunes et al. (2000), Aydin and Sevinc (2006) and Hosseini et al. (2007). Yau (2010) stated that the physiology and mechanisms of boron toxicity are not well understood. However, B uptake is closely related to the B concentration of the soil solution and the rate of water transpiration.

When B concentration in soil solution is large, B is distributed throughout the plant in normal transpiration stream, causing the accumulation of B in leaf margins and leaf tips. Excessive amounts of B appear to inhibit the formation of starch from sugars or results in the formation of carbohydrate complexes, and failing in grain filling. The reason of boron toxicity is still unclear, but, the complex-forming ability of boron may be the cause of this toxicity (Nuttall, 2000, Brown et al. 2002; Bolanos et al. 2004;

Camacho-Cristobal et al. 2008). The inhibition of barley growth caused by application of 3 mg L⁻¹ B was improved at different levels by application of some plant growth modulators (Table 4). A higher leaf area coupled with higher contents of Chl a and Chl b as well as increased yield parameters were detected in the presence of different concentrations of the applied growth modulators in plants exposed to 3 mg L⁻¹ B application (Table 5). This reveals the efficiency of these modulators to ameliorate the toxic effect of B on barley growth and yield. The ameliorative effect of glutamic acid and glycine on barley growth could be explained as these amino acids were shown to have a role in inducing tolerance under drought stress in cotton, rice and maize (Thakur and Rai, 1982; Yang et al., 2000). In epidermal cells of *Vicia faba* leaf, some amino acids including glutamic acid stimulated stomatal opening leading to promote the K⁺ influx into the guard cells. Glycine-rich proteins have been found in the cell walls in many higher plants, which form a third group of structural protein components of the wall (Ringlia et al 2001). The alleviation of inhibitory effect of B treatment using ascorbic acid could be attributed to its role as an antioxidant against reactive oxygen species that are formed from photosynthetic and respiratory processes (Pastori et al., 2003).

Foliar application of ascorbic acid was found to have positive effects on growth parameters in lemon grass (Tarraf et al., 1999), enhance photosynthesis in soybean (Gloden-Goldhirsh et al., 1995), and increase macronutrient contents (N, P and K) in sweet pepper (Talaat, 2003). Application of 3 mg L⁻¹ B only induced the accumulation of B, N, P and K in barley shoots and roots compared to those determined in presence of SA or CaCl₂ (Table 6). Higher accumulation of B in barley shoots than roots in the present study is in close agreement with the findings of many investigators who demonstrated that B is taken up by the roots in the form of boric acid and transported through the xylem to the shoots. Thus, B concentrations in roots remain relatively low compared to those in leaves (Nable et al., 1997).

In the present study, the high ion content of barley tissues in response to addition of B is in agreement with the results obtained by many researchers such as Eraslan et al. (2008, 2007b) and Koohkan et al. (2008). There are a number of reported interactions between B and the other nutrients. Shelp and Shattuck (1987a, b) reported decrease of re-translocation of Cu, N, and Zn to sink tissues. However, overall increase of P under increment of B concentration suggests a promoted remobilization of P in plant tissues.

In agreement with our results, Wimmer et al. (2003) indicated that the slight tendency toward higher intercellular soluble K concentrations might indicate some membrane damage resulting in higher K leakage into the apoplasmic space. Moreover, B functions in the regulation of plant membranes and ATPase of corn is a possible component of transport processes leading to a reduced capacity for the absorption of phosphate (Tariq and Mott, 2007). Also, effects of boron and membrane permeability could lead to association between B and K. The stimulation of K accumulation by ATPase proton pumps which may account for positive correlation between K and B recommended by Shorrocks (1990). Therefore, injured plasma membranes of barley tissues under excess B (3 mg L⁻¹) stress in our experiments may lose their control mechanism on the diffusions of ions as evidenced by a high influx of K, N and P. However, our results clearly demonstrated that B treated barley tissues responded positively to the five tested alleviators at the following sequence: salicylic acid >calcium

Table 4. Effect of different concentrations of glutamic acid (GA), glycine (Gly), ascorbic acid (AA), salicylic acid (SA) and calcium chloride (CaCl₂) on shoot and root fresh weight (g plant⁻¹) of 3.0 (mg L⁻¹)B treated barley at vegetative and flowering stages.

Treatment	Shoot				Root			
	Fresh weight		Dry weight		Fresh weight		Dry weight	
	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering
3.0 mg/L B	2.24 ± 0.20	3.08 ± 0.24	0.335 ± 0.0	0.42 ± 0.03	0.16 ± 0.002	0.48 ± 0.02	0.030 ± 0.001	0.080 ± 0.00
+ 1.0 mM GA	2.25 ± 0.13 ns	3.08 ± 0.22 ns	0.336 ± 0.01 ns	0.42 ± 0.02 ns	0.16 ± 0.003 ns	0.48 ± 0.03 ns	0.030 ± 0.001 ns	0.080 ± 0.00 ns
+ 3.0 mM GA	2.26 ± 0.2 ns	3.10 ± 0.15 ns	0.338 ± 0.0 ns	0.42 ± 0.01 ns	0.17 ± 0.003*	0.48 ± 0.03 ns	0.030 ± 0.001 ns	0.080 ± 0.00 ns
+ 1.0 mM Gly	2.32 ± 0.33 *	3.20 ± 0.22 *	0.346 ± 0.02 **	0.44 ± 0.03 **	0.17 ± 0.006*	0.50 ± 0.04**	0.030 ± 0.001 ns	0.082 ± 0.00**
+ 3.0 mM Gly	2.28 ± 0.13 ns	3.14 ± 0.25 ns	0.340 ± 0.0 ns	0.43 ± 0.02**	1.17 ± 0.004*	0.49 ± 0.04 ns	0.030 ± 0.001 ns	0.080 ± 0.00 ns
+ 2.0 mM AA	2.34 ± 0.4 **	3.20 ± 0.20 **	0.350 ± 0.01**	0.44 ± 0.02 **	0.17 ± 0.003 *	0.53 ± 0.03 **	0.030 ± 0.001 ns	0.082 ± 0.00**
+ 5.0 mM AA	2.40 ± 0.33**	3.25 ± 0.30 **	0.357 ± 0.01**	0.46 ± 0.01 **	0.17 ± 0.002 *	0.53 ± 0.02 **	0.031 ± 0.002*	0.089 ± 0.00**
+ 1.0 mM SA	2.64 ± 0.27**	4.10 ± 0.26 **	0.394 ± 0.01 **	0.56 ± 0.02 **	0.20 ± 0.001 **	0.64 ± 0.02 **	0.040 ± 0.001**	0.113 ± 0.00**
+ 5.0 mM SA	2.52 ± 0.54**	3.87 ± 0.26**	0.376 ± 0.01 **	0.53 ± 0.02 **	0.18 ± 0.004 **	0.59 ± 0.02 **	0.032 ± 0.002**	0.098 ± 0.00**
+ 1.0 mM CaCl ₂	2.46 ± 0.13**	3.78 ± 0.23 **	0.368 ± 0.02 **	0.52 ± 0.02 **	0.18 ± 0.002 **	0.58 ± 0.04 **	0.033 ± 0.001**	0.096 ± 0.00**
+ 5.0 mM CaCl ₂	2.61 ± 0.2**	4.14 ± 0.20 **	0.390 ± 0.0**	0.57 ± 0.01**	0.20 ± 0.001 **	0.65 ± 0.02 **	0.040 ± 0.001**	0.110 ± 0.00**
L.S.D 0.05	0.06	0.12	0.01	0.01	0.01	0.01	0.001	0.002
L.S.D 0.01	0.08	0.22	0.01	0.01	0.01	0.02	0.01	0.01

* Results significantly different from control at (P < 0.05). ** Results significantly different from control at (P < 0.01). ns: non significant

Table 5. Effect of different concentrations of glutamic acid (GA), glycine (Gly), ascorbic acid (AA), salicylic acid (SA) and calcium chloride (CaCl₂) on leaf area, Chl a and Chl b of 3.0 mg/L B treated barley at vegetative and flowering stages.

Treatment	Leaf area (cm ²)		Chl a (mg/cm ²)		Chl b (mg/cm ²)	
	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering
	3.0 mg/L B	3.55 ± 0.03	16.44 ± 1.00	40.0 ± 2.1	86.1 ± 1.8	19.5 ± 0.57
+ 1.0 mM GA	3.56 ± 0.03 ns	16.47 ± 0.51 ns	40.1 ± 1.3 ns	86.3 ± 1.2 ns	19.5 ± 0.89 ns	39.2 ± 1.2 ns
+ 3.0 mM GA	3.58 ± 0.03 ns	16.52 ± 0.92 ns	40.2 ± 1.2 ns	86.4 ± 2.0 *	19.7 ± 0.89 ns	40.1 ± 2.3 *
+ 1.0 mM Gly	3.85 ± 0.05 **	18.60 ± 0.97 **	41.5 ± 1.0 ns	86.2 ± 2.2 ns	20.9 ± 0.94 *	40.5 ± 1.1 **
+ 3.0 mM Gly	3.61 ± 0.06 ns	17.29 ± 1.10 ns	40.9 ± 1.1 ns	86.1 ± 2.2 ns	19.9 ± 0.97 ns	41.1 ± 2.1 **
+ 2.0 mM AA	3.80 ± 0.04 **	19.19 ± 1.00 **	42.6 ± 2.0 ns	88.0 ± 1.8 **	22.2 ± 1.10 *	42.2 ± 2.7 **
+ 5.0 mM AA	3.95 ± 0.07 **	19.11 ± 0.22 **	42.0 ± 1.6 ns	88.0 ± 1.7 **	22.2 ± 1.00 *	43.0 ± 2.3 **
+ 1.0 mM SA	4.45 ± 0.02 **	23.21 ± 0.87 **	46.2 ± 1.8 **	91.2 ± 2.1 **	25.2 ± 0.82 **	44.8 ± 1.7 **
+ 5.0 mM SA	3.87 ± 0.07 **	20.98 ± 0.93 **	44.2 ± 1.5 **	89.5 ± 1.7 **	22.9 ± 1.00 **	42.9 ± 2.1 **
+ 1.0 mM CaCl ₂	3.96 ± 0.08 **	20.25 ± 1.50 **	43.3 ± 1.5 *	90.2 ± 2.1**	22.9 ± 0.98 **	42.8 ± 1.3 **
+ 5.0 mM CaCl ₂	4.43 ± 0.04 **	23.35 ± 0.78 **	46.5 ± 1.4 **	92.1 ± 1.3**	24.9 ± 0.79 **	44.9 ± 1.2 **
L.S.D 0.05	0.08	1.54	2.61	0.28	2.5	0.96
L.S.D 0.01	0.11	2.09	3.54	0.49	3.1	1.87

* Results significantly different from control at (P < 0.05). * Results significantly different from control at (P < 0.01). ns: non significant

Table 6. Mineral content (%) of 3.0 mg/L B treated barley in presence of SA or CaCl₂ at various growth stages.

Stage	Treatment	Boron (%)		Nitrogen (%)		Phosphorus (%)		Potassium (%)	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Vegetative	3.0 mg/L B	9.4 ± 0.81	3.0 ± 0.34	5.3 ± 0.33	4.4 ± 0.41	0.54 ± 0.02	0.68 ± 0.03	6.2 ± 0.31	0.75 ± 0.04
	+ 1.0 mM SA	7.1 ± 0.75 **	2.3 ± 0.30 **	3.6 ± 0.31 **	2.8 ± 0.32 **	0.37 ± 0.02 **	0.44 ± 0.02 **	4.3 ± 0.25 **	0.51 ± 0.01 **
	+ 5.0 mM CaCl ₂	7.1 ± 0.72 **	2.3 ± 0.18 **	3.6 ± 0.21 **	2.8 ± 0.30 **	0.36 ± 0.02 **	0.44 ± 0.01 **	4.3 ± 0.24 **	0.51 ± 0.01 **
	L.S.D 0.05	1.43	0.29	0.52	0.62	0.03	0.036	0.49	0.04
	L.S.D 0.01	2.07	0.42	0.75	0.89	0.05	0.051	0.71	0.06
Flowering	3.0 mg/L B	13.9 ± 0.89	4.5 ± 0.36	5.5 ± 0.53	4.9 ± 0.51	0.56 ± 0.03	0.60 ± 0.02	6.6 ± 0.40	1.64 ± 0.12
	+ 1.0 mM SA	9.8 ± 0.92 **	3.2 ± 0.17 **	3.4 ± 0.30 **	2.8 ± 0.32 **	0.33 ± 0.02 **	0.34 ± 0.01 **	3.9 ± 0.25 **	1.0 ± 0.01 **
	+ 5.0 mM CaCl ₂	9.7 ± 0.46 **	3.2 ± 0.16 **	3.4 ± 0.21 **	2.8 ± 0.31 **	0.33 ± 0.02 **	0.34 ± 0.01 **	3.9 ± 0.24 **	1.0 ± 0.01 **
	L.S.D 0.05	1.46	0.43	0.70	0.7	0.04	0.03	0.55	0.12
	L.S.D 0.01	2.12	0.62	1.02	0.1	0.06	0.04	0.79	0.17
Harvesting	3.0 mg/L B	18.5 ± 1.03	6.8 ± 0.72	5.0 ± 0.46	5.13 ± 0.42	0.52 ± 0.02	0.91 ± 0.03	7.6 ± 0.39	0.57 ± 0.03
	+ 1.0 mM SA	12.0 ± 1.11 **	4.7 ± 0.63 **	3.0 ± 0.25 **	3.10 ± 0.36 **	0.31 ± 0.01 **	0.52 ± 0.02 **	4.5 ± 0.38 **	0.34 ± 0.02 **
	+ 5.0 mM CaCl ₂	11.9 ± 0.99 **	4.6 ± 0.59 **	3.0 ± 0.35 **	3.10 ± 0.34 **	0.31 ± 0.01 **	0.52 ± 0.02 **	4.5 ± 0.36 **	0.34 ± 0.02 **
	L.S.D 0.05	1.94	1.2	0.57	0.67	0.025	0.04	0.73	0.05
	L.S.D 0.01	2.82	1.7	0.83	0.98	0.04	0.06	1.06	0.07

* Results significantly different from control at (P<0.05). ** Results significantly different from control at (P<0.01). ns : non significant

chloride > ascorbic acid > glycine > glutamic acid. Interestingly, treatment with salicylic acid or calcium chloride induced almost the same ameliorative effect on growth of B treated barley. Increasing salicylic acid concentration from 1 mM to 5 mM inhibited its ameliorative effect while increasing calcium chloride concentration from 1 mM to 5 mM stimulated its ameliorative effect. The dose dependent biphasic action of SA was shown also by Sahu et al (2010) in wheat concerning phosphate uptake. Therefore, the ameliorative impact of both salicylic acid (1 mM) and calcium chloride (5 mM) on 3 mg L⁻¹ B treated barley growth stimulate more extensive investigation in lab experiments.

Materials and methods

Plant material

Barley grains (*Hordeum vulgare* L. cv. Giza 123) were obtained from Sakha research Station Kafr El-Shaikh Governorate. The grains (30 grains) were surface sterilized and germinated in pots filled with sandy soil (pH 7.5 and B concentration 0.4 mg L⁻¹). All pots were irrigated with distilled water for two weeks, then Hoagland solution (2.5 mM Ca(NO₃)₂, 3.0 mM KNO₃, 0.17 mM KH₂PO₄, 1.5 mM MgSO₄, 50 µM Fe as (Na Fe DTPA), µM MnSO₄, 0.4 µM ZnSO₄, 0.2 µM CuSO₄ and 0.1 µM H₂ MoO₄) was added twice a week.

Boron treatment

The effect of boron on barley growth was studied by using elevated B concentrations of 0, 0.5, 1.5, 3.0 and 6.0 mg L⁻¹ (as boric acid added to Hoagland solution). The efficiency of some growth modulators in alleviating the growth inhibition was evaluated under B stress. The following substances were added to Hoagland solution in the presence of 3.0 mg L⁻¹ B: glutamic acid (1 or 3 mM), glycine (1 or 3 mM), ascorbic acid (2 or 5 mM), salicylic acid (1 or 5 mM) and calcium chloride (1 or 5 mM).

Experimental design and data analysis

Barley grains were cultivated and left to grow till the end of the season. Samples were collected at vegetative (22-day-old), flowering (45-day-old) and harvesting stages (92-day-old). Three sets of treatments were prepared for the different measurements. In the set I, plants irrigated with Hoagland solution without B addition; set II, plants irrigated with Hoagland solution with the addition of different B concentrations and set III, plants irrigated with Hoagland solution with the addition of different concentrations of the above mentioned plant growth modulators in the presence of 3.0 mg L⁻¹ B. Each experiment was set up as randomized complete block (RCBD) with 3 replicates each containing a row of all treatments. Samples from each treatment were collected and subjected to the different measurements. Data was statistically analyzed using Analysis of variance (ANOVA) for RCBD followed by the computation of least significant difference (LSD) at p ≤ 0.5 and 0.01 levels according to Cochran and Cox (1960).

Growth parameters

Samples harvested at different growth stages (vegetative, flowering and harvesting) were weighed to determine their fresh weights, then oven-dried at 70°C for 2 days and reweighed to determine the dry weights. Both fresh and dry weights were estimated as gm individual⁻¹. Leaf area (cm²) was calculated at vegetative (22-day-old) and flowering (45-day-old) stages using portable area meter model Li-3000A Li-COR. Weight of both straw and grains were estimated as g per 20 individuals.

Photosynthetic pigments

The photosynthetic pigments (Chl a and Chl b) were estimated in barley leaves at vegetative and flowering stages using the Arnon (1949) proposed approach.

Mineral nutrient contents

The powdered oven dry plant materials (shoots, roots and grains) were exposed to acid digestion as follows: 0.2 g of plant sample was digested in 5 ml of H₂O₂ and 1 ml HClO₄ as described by Chapman and Pratt (1961). The extract of digested plant materials was used for the determination of mineral nutrient contents as follow: Nitrogen (N) content by micro-Kjeldahl (Page, 1982), Total phosphorus (P) content by spectrophotometer (Snell and Snell, 1967), potassium (K) content using flame photometer (Jackson, 1967), and boron content according to John et al. (1975).

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