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Boron supply alleviates Al-induced inhibition of root elongation and physiological characteristics in rapeseed (*Brassica napus* L.)

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ABSTRACT

Aluminum (Al) toxicity is one of the major problems affecting crop production. Boron (B) is an essential micronutrient for higher plants. In the present study, we investigated the alleviation of Al-induced inhibition of root growth and physiological characteristics by B in rapeseed. The rapeseeds were grown in different Al concentrations (0 and 300 µM), and for every concentration, two B treatments (2.5 and 25 µM as H_3BO_3) were applied. The results showed that Al toxicity under low B drastically inhibited root growth. The supply of B improved root length, photosynthesis, root activity, total chlorophyll by 60.15%, 104.7%, 102%, and 106.3%, respectively under Al toxicity. This further resulted in improvement of peroxidase, catalase, and ascorbate peroxidase activities while decreasing malondialdehyde, H_2O_2, and Al contents in roots and leaves. It might be supposed that B alleviates Al toxicity by less mobilization of Al in plant parts and through improving antioxidant enzyme activities.

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KEYWORDS
Al toxicity; boron; physiology; root morphology; antioxidant defense system

Introduction

Aluminum (Al) is one of the most abundant elements in the earth's crust, (after oxygen and silicon). It is usually present as an insoluble, less hazardous alumino-silicates or Al oxides, that can easily become soluble under acidic conditions, and may induce toxicity issues in plants (Poschenrieder et al. 2008). Moreover, Al^{3+} under acidic condition can easily be taken up by susceptible plants (George et al. 2012). It has long been reported by various studies that Al toxicity could result in inhibition of root growth. Al-induced inhibition of root growth is the first symptom and becomes apparent within minutes of Al exposure even at a negligible concentration (Kochian 1995; Pineros and Kochian 2001; Doncheva et al. 2005). Blevins (1987) proposed that Al toxicity might cause root inhibition by inducing Boron (B) deficiency due to similarities in symptoms of Al stressed and B-deficient plants. Lukaszewski and Blevins (1996) while studying on B-deficient or Al toxic squash plants, proposed that the inhibition of root growth could be a result of a disrupted ascorbate metabolism. Moreover, B-deficiency increased the concentration of un-methylated pectin in the cell wall of roots, creating a more favorable condition for the binding of Al (Stass et al. 2007). Additionally, abundant rainfall washes out basic cations from the root zone favoring acidic environment. In acidic conditions, B can be easily leached from the soils, producing B-deficiency problems (Shorrocks 1997). However, the exact mechanism of root growth inhibition has not been elucidated yet. Some researchers have reported that Al can be beneficial for plant growth in some plants (Foy and Fleming 1982) by activating antioxidant enzymes, enhancing root growth, stimulating leaf growth (Ghanati et al. 2005; Wang et al. 2015).

Boron (B) is an essential micronutrient (H_3BO_3, boric acid) for the normal development and growth of higher plants. The role of B has been well reported in the establishment of the primary cell wall by cross-linking with pectic polypectic polysaccharide RG-II (rhamnogalacturonan II). This cross-linked RG-II gives rise to stable, complex cell wall structure with reduced pore spaces. So this complex network of cells makes the cell wall tighter and hinders the entrance of Al into the cell, thus preventing Al contact with cell organelles (O'Neill et al. 2004; Corrales et al. 2008). Amelioration of Al toxicity by using B has been stated by many researchers in squash (LeNoble et al. 1996b), alfalfa (LeNoble et al. 1996a), sour pummel (Jiang et al. 2009), pea (Yu et al. 2009), sunflower (Ruiz et al. 2006), common bean (Stass et al. 2007), rapeseed (Yan et al. 2018), trifoliate (Riaz et al. 2018a, 2018b) and however, such an effect was not found by Wang et al. (2005) in maize. Many reports have indicated the mechanism behind B-induced alleviation of Al-toxicity, yet the actual mechanism has not been clarified. Some researchers proposed that B application prevented binding sites for Al, and reduced Al direct contact with roots (Stass et al. 2007; Yu et al. 2009), and this lower Al uptake prevents in the decrease of photosynthesis and alleviates the chlorosis symptoms of Al toxicity (Jiang et al. 2009; Yu et al. 2009).

In China, rapeseed (*Brassica napus* L.) is the fourth largest crop after rice, maize, and wheat. It accounts for about 20% of world production and it is usually grown for its oil-rich seed and is regarded as third main vegetable oil source. Amelioration of Al toxicity by the addition of B has been reported by many researchers. However, none of the studies have previously investigated the effect of B application in rapeseed plants suffering from Al toxicity. Keeping in view, a study was conducted to investigate whether B supply could alleviate Al-induced inhibition of root elongation and physiological characteristics in rapeseed. Moreover, measuring how the B could induce activation of

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antioxidant enzyme system that may contribute to alleviate Al effects on H_2O_2 and MDA levels.

Materials and methods

Experimental condition and plant materials

The experiment was carried out at Huazhong Agriculture University, Wuhan, China. The seedlings of rape were grown under hydroponic culture in black boxes containing 4 liters of nutrient solution in a growth chamber at 16 h light/8 h dark photoperiod and a 22°C day/20°C night temperature regime at 75% relative humidity. The nutrient solution was applied as; 0.51 g L^{-1} KNO_3, 1.18 g L^{-1} Ca $(NO_3)_2.4H_2O$, 0.49 mg L^{-1} $MgSO_4.7H_2O$, 0.14 mg $L^{-1}K_2$ HPO_4, 0.075 mg L^{-1} $CuSO_4.5H_2O$, 1.78 mg L^{-1} $MnCl_2.4H_2O$, 0.23 mg L^{-1} $ZnSO_4.7H_2O$, 0.025 mg L^{-1} $Na_2MoO_4.2H_2O$, and 10 mg L^{-1} Fe-EDTA. The nutrient solution was applied in one-fourth strength for a week before starting B and Al treatments, and then the solution strength was gradually increased to full strength. The pH was maintained at 4.0 by NaOH and HCl. All black pots were washed with 0.1 M HCl prior to transplanting rapeseed seedlings. Treatments containing different Al concentrations (0 and 300 µM as $AlCl_3{\cdot}6H_2O$), and for every concentration two B treatments (2.5 and 25 µM as H_3BO_3) in 0.5 mM $CaCl_2$ were applied in complete randomized design. Al and B speciation in the solutions was performed with GEOCHEM-PC programe. The nutrient solution was replaced with a fresh solution on alternate days. After 29 days (Figure 1), the plants were harvested. The plant parts (leaf, root, and stem) were individually sampled after washing with deionized water and immediately kept at −80°C until used for further determination of enzymes. One part of samples were dried at 40°C until a constant weight for later analysis of B.

Measurement of photosynthetic traits and chlorophyll contents

Leaf gas exchange traits viz. photosynthesis (*Pn*), transpiration rate (*E*) and stomatal conductance (*gs*) were measured by utilizing portable photosynthesis system (infrared gas analyzer, Li-COR 6400 XT, USA) in a daytime during 10–12 o'clock one day before harvesting of the plants. For the accuracy of photosynthetic readings, three healthy, undamaged and fully expanded leaves from the same plant were selected at the top of the plant. The plants were randomly selected from each replication. The leaf chlorophyll contents (photosynthetic pigments) were determined by taking 0.2 g fresh leaves after carefully washing them with de-ionized water to remove any contamination. The leaves were chopped into small pieces and treated with 95% ethanol for 24 h in dark. The absorbance values were recorded at 649 nm (chlorophyll b), 665 nm (chlorophyll a), and 470 nm (carotenoids) with a spectrophotometer and total chlorophyll contents were expressed as (mg g^{-1} FW).

Figure 1. Rapeseed seedlings growth under different Al and B concentrations. −Al−B (2.5 µM B with 0 µM Al), ++Al+B (2.5 µM B and 300 µM Al), −Al ++B (25 µM B with 0 µM Al as control) and ++Al ++B (25 µM B and 300 µM Al).

Measurement of root morphological characteristics and root activity

At the end of the experiment, the plant roots were scanned to study different root related morphological characteristics i.e. root length (cm), surface area (cm^2), average diameter (mm) and total root volume (cm^3) by using WinRHIZO software supported by root scanner (Epson Perfection V-700). The root activity was measured according to TTC (triphenyl tetrazolium chloride) reduction method (Islam et al. 2007). The roots after harvesting were thoroughly washed with de-ionized water. The 0.5 g roots were treated with 5 mL TTC (triphenyl tetrazolium chloride) (0.4%) and 0.1 mM (pH 7.0) phosphatic buffer. After two hours incubation at 37°C, 1 *M* H_2SO_4 was used to terminate the reaction. Finally, these roots were treated with methanol and incubated at 37°C until all color washed-out. The absorbance was recorded at 485 nm by spectrophotometer and stated as the quantity of triphenyl formazan (TPF) de-oxidized by triphenyl tetrazolium chloride (TTC), and was expressed as TPF (µg^{-1} mg fresh root h^{-1}).

Histochemical determination of Al by hematoxylin staining

For hematoxylin root staining, rapeseed roots were randomly chosen from different B and Al treatments. The selected roots were stained by the Yu et al. (2009) method. After soaking in a solution of 0.2% hematoxylin and 0.02% KI, the plant roots were washed with de-ionized water until no dye in the rinsing water and studied under stereomicroscope and images were taken with Nikon digital camera.

Determination of Al and B in root tips and leaves

For determination of Al concentrations in leaves and roots (0–10 mm root apices), the roots were washed 3 times with 0.5 mM $CaCl_2$ solution, and Al concentrations were determined by graphite furnace atomic absorption spectrophotometer (GTA 120 Graphite Tube Atomizer, Agilent Technologies, 200 series AA) as described by Yu et al. (2009). B contents in leaves and roots were measured by Dible et al. (1954) method. Briefly, 0.2 g powdered sample of roots and leaves were ashed at 550°C for 4 h and treated with 0.1 M HCl. The absorbance was recorded at 540 nm by a spectrophotometer.

Determination of MDA, H_2O_2 contents and antioxidant enzyme activities

For measurement of enzymes like superoxide dismutase activity (SOD), peroxidase activity (POD), ascorbate peroxidase activity (APX), and catalase activity (CAT), 0.5 g fresh leaves were homogenized in sodium phosphate buffer solution (PBS, pH = 7.8) with pre-cold mortar and pestle at 4° C, the homogenized leaf samples were centrifuged at 12000 × g for 10 min, and supernatant was collected in another tube and referred to enzyme extract. The spectrophotometer was used to measure optical density (OD) at different absorbance.

The superoxide dismutase activity was measured by Beauchamp and Fridovich (1971) method, the ability of SOD to restrict the photochemical reduction of NBT (nitro tetrazolium blue chloride). Briefly, the reaction mixture (total 3 mL) containing 0.1 mol/L PBS (phosphate buffer solution, pH = 7.8), 1.3 mol/L methionine, 0.3 mL (750 µmol/L) NBT, 0.3 mL (100 µmol/L EDTA-Na^2), 0.25 mL distilled water, 0.3 mL riboflavin and 0.05 mL (EE) enzyme extract while 0.05 mL PBS was used instead of enzyme extract as a control tube. All these samples were placed under light 4000 flux for 20 min at 25°C and control tube in dark. The absorbance was recorded at 560 nm. The peroxidase (POD) activity was measured by Cai et al. (2008) method by an increase in absorbance. Briefly, the reaction mixture (total 3.0 mL) contained 1.0 mL of (0.3%) H_2O_2 solution, PBS (0.05 M, pH = 7.0), 0.2% guaiacol, and 50 µL enzyme extract. The POD activity was determined spectrophotometrically over 3 min at 470 nm. The catalase activity (CAT) was determined by Aebi (1983) method by a decrease in absorbance. Briefly, the reaction mixture (total 3.0 mL) containing 2.55 mL deionized water, 400 µL (0.3%) H_2O_2 and 50 µL enzyme extract and absorbance was recorded by spectrophotometer at 240 nm for 3 min. The ascorbate peroxidase activity (APX) was measured according to Nakano and Asada (1981) method by a decrease in absorbance. Briefly, 3.0 mL reaction mixture containing 0.05 M phosphate buffer (PBS, pH 7.0), ascorbate (0.5 mM ASA), 50 µL enzyme extract and 0.6 mM H_2O_2. The absorbance was recorded by spectrophotometer at 290 nm. The malondialdehyde (MDA) concentration in the rapeseed was measured by Vos et al. (1991) method. The 0.5 g leaves were ground in N_2 and 5% trichloroacetic acid (TCA) and were centrifuged for 15 min at 12000 × g. The supernatant was collected in another tube. The reaction mixture was allowed to react at 98°C for 20 min and finally cooled in ice bath. The reaction mixture containing 2 mL 0.5% TBA solution, 0.5 mL PBS solution (pH = 7.0) and 1.5 mL enzyme liquid were centrifuged again. The MDA absorbance was measured at 600, 450, and 532 nm by a spectrophotometer. The contents of H_2O_2 were measured according to potassium iodide method (Velikova et al. 2000). The leaves (0.1 g) of rapeseed were homogenized in 0.1% TCA and centrifuged at 12000 × g for 20 min. The reaction mixture consisted of 10 mM potassium phosphate, 1 M KI and 0.5 mL of supernatant. The absorbance was observed spectrophotometrically at 390 nm.

Statistical analysis

All collected data were statistically evaluated by analysis of variance (ANOVA) employing analytical software (Statistix 8.1, Tallahassee, FL, USA). A two-way analysis of variance (ANOVA) was carried out, and the mean values were compared using Duncan's multiple range test.

Results

Effect of B and Al on root morphological characteristics

The results showed that under low B, the Al toxicity severely inhibited root length, average diameter, total root volume and

Table 1. Effect of Al and B on root morphology and root activity contents of rapeseed.

Treatments	Root length (cm)	Surface area (cm^2)	Avg. diameter (mm)	Root volume (cm^3)	Root activity µg mg^{-1} fresh root h^{-1}
−Al−B	$20.75^{c} \pm 0.33$	$178^{b} \pm 3.89$	$0.55^{bc} \pm 0.02$	$1.67^{c} \pm 0.02$	$381^{b} \pm 9.38$
−Al−++B	$27.05^{a} \pm 1.2$	$260^{a} \pm 6.07$	$0.76^{a} \pm 0.02$	$4.95^{a} \pm 0.13$	$457^{a} \pm 16.95$
+Al−B	$14.7^{d} \pm 0.78$	$117^{c} \pm 4.12$	$0.47^{b} \pm 0.00$	$1.57^{c} \pm 0.08$	$222^{c} \pm 9.32$
+Al+++B	$23.54^{b} \pm 0.26$	$253^{a} \pm 20.83$	$0.76^{b} \pm 0.02$	$4.18^{b} \pm 0.2$	$449^{ab} \pm 14.25$
B	**	**	**	**	**
Al	**	**	ns	**	**
B × Al	*	*	*	*	*

Note: *−Al-B (2.5 µM B with 300 µM Al), ++Al-B (2.5 µM B and 300 µM Al), µ−Al+B (25 µM B with 0 µM Al as control) and, µ ++Al+B (25 µM B and 300 µM Al). Different letters (a, b, c, d) in a column indicate significant differences among 4 treatments at 95% probability level with ± S.E; *, ** indicates significance at the $p < 0.05$ and $p < 0.01$ respectively; ns indicates non-significance.

Table 2. Effect of Al and B interactions on DW of root and shoot, and the concentrations of Al and B in root and leaf of rapeseed.

Treatments	Root DW mg/plant	Shoot DW g/plant	Root B mg/kg DW	Leaf B mg/kg DW	Root tip Al mg/root	Leaf Al mg/g
−Al−B	144.25c ± 2.02	1.77c ± 0.06	$4.26^{b} \pm 0.22$	$7.47^{b} \pm 0.39$	$0.14^{c} \pm 0.01$	$0.14^{c} \pm 0.01$
−Al−++B	229.72a ± 7.58	3.28a ± 0.16	$25.13^{a} \pm 1.21$	$37.06^{a} \pm 1.79$	$0.12^{c} \pm 0.01$	$0.13^{c} \pm 0.01$
+Al−B	124.05c ± 0.99	1.41d ± 0.02	$5.7^{b} \pm 0.21$	$7.95^{b} \pm 0.17$	$1.41^{a} \pm 0.08$	$2.02^{a} \pm 0.09$
+Al+++B	191.65b ± 7.37	2.58b ± 0.08	$27.31^{a} \pm 1.08$	$35.3^{a} \pm 0.97$	$0.67^{b} \pm 0.04$	$1.32^{b} \pm 0.07$
B	**	**	**	**	**	**
Al	**	**	ns	ns	**	**
B × Al	*	*	ns	ns	**	**

Note: DW: dry weight; Shoot: Leaf and stem; *−Al-B (2.5 µM B with 300 µM Al), µ ++Al-B (2.5 µM B and 300 µM Al), µ−Al+B (25 µM B with 0 µM Al as control) and, + ++Al+B (25 µM B and 300 µM Al). Different letters (a, b, c, d) in a column indicate significant differences among 4 treatments at 95% probability level with ± S.E; *, ** indicates significance at the $p < 0.05$ and $p < 0.01$ respectively; ns indicates non-significance.

surface area (Table 1). However, in the presence of Al, B supply (25 µM B) significantly increased root length, average diameter, total root volume and surface area by 60.15%, 61.34%, 166.36% and 114.34% respectively compared to low B treatment. Moreover, under low B, Al toxicity inhibited the root activity (Table 2) and the supply of 25 µM B significantly increased the root activity by 101.96% under Al toxicity compared to low B treatment. It shows that the addition of B had a positive role in the amelioration of root activity under Al stress.

Effect of Al and B on the root, shoot dry weight and concentrations of B and Al

Compared to the B treated plants without Al, the treatment of low B with or without Al decreased root and shoot dry weights and the effects were more pronounced under Al treatment. However, B (25 µM) treatment significantly increased the root and shoot dry weights by 54.49%, 82.33%, with Al respectively. The results showed that Al concentrations in the roots and leaves increased under both B treatments, and Al concentration was more pronounced in the leaves than roots (Table 2). Al treated root and shoot had significant higher Al concentration in the + Al−B than + Al + B treatment (Table 2). In the absence of Al, leaf and root Al did not significantly change in response to both B treatments. However, Al concentration in the Al-treated roots and leaves had significantly decreased by 25 µM B. The B concentrations in leaves and roots increased with increasing B concentrations, and more B concentration was found in 25 µM B than 2.5 µM B treatment under Al stress. The results showed that comparatively more B was found in leaves than in roots. In the Al-treated rapeseed, the Al did not cause significant effects on B uptake. According to GEOCHEM speciation, most (90%) of the Al was present as a free metal. B concentrations was found complexed with H. Al supply had little effect on B speciation. In all Al treatments, more than 97% of B remained complexed by H, and less than 3% of B was complexed by Al.

Effect of B and Al on leaf gas exchange traits and total chlorophyll contents

Al toxicity with 2.5 µM B significantly inhibited photosynthesis (*Pn*), stomatal conductance (*gs*), transpiration rate (*E*) and total chlorophyll contents compared with −Al + B treatment. Furthermore, low B treatment without Al also inhibited leaf gas exchange traits but the effects were less pronounced than those recorded under + Al−B (Table 3). Compared to the low B treatment, the supply of 25 µM B significantly increased *Pn*, *gs E* and total chlorophyll by 104.54%, 74.93%, 63.65% and 1.6.32% respectively under Al toxicity.

Effect of B and Al on hematoxylin staining of roots

The sensitivity of hematoxylin staining to Al-toxicity is a fast method for revealing Al in root apices. Our results exhibited that Al was obviously detected by hematoxylin staining in the root apex with dense blue color under low B (Figure 2(c)). The intensity of dye showed that Al invaded the root tips and penetrated into central cylindrical part of the root. Hematoxylin dying revealed that upon the supply of 25 µM, less Al was detected in the root tips (Figure 2(d)). Our results showed that irrespective of the B concentration, plants roots without Al did not stain, and Al was not detected by hematoxylin, showing clean and bright roots (Figure 2(a,b)).

Effect of B and Al on the contents of MDA and H_2O_2, and antioxidant enzyme activities

Significant variations in the activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), H_2O_2 and malondialdehyde (MDA) contents were observed in response to Al under different B concentration. Under low B, Al toxicity resulted in higher stress, and to lower down this stress, SOD activity was found to be triggered in the leaves (Figure 3). Moreover, Al-toxicity inhibited the activities of the POD, CAT and APX antioxidant enzyme activities compared to −Al + B treatment (Figure 3). Compared to the + Al−B, the supply of 25 µM B reduced the SOD activity and significantly increased POD, CAT and APX activities by 54.7%, 87.3%

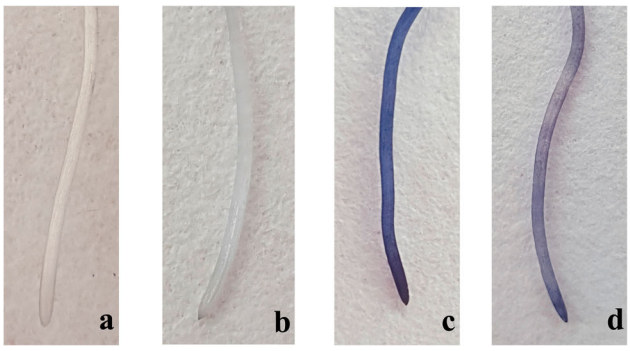

Figure 2. Histochemical determination of Al by hematoxylin staining of the rapeseed roots. Roots were stained by hematoxylin containing potassium iodide. (a) Plants treated with 2.5 µM B without Al (−Al−B), (b) treated with 25 µM B without Al (−Al−++B), (c) treated with 2.5 µM B with 300 µM Al (++Al−B) and (d) treated with 25 µM B and 300 µM Al (++Al ++B).

Table 3. Effect of Al and B on leaf gas exchange parameters and total chlorophyll contents of rapeseed.

Treatments	Photosynthesis µmol $m^{-2}s^{-1}$	Stomatal conductance mmol m^{-2} s^{-1}	Transpiration rate mmol m^{-2} s^{-1}	Total chlorophyll mg g^{-1} FW
−Al−B	$10.84^{c} \pm 0.31$	$317^{b} \pm 19.66$	$4.89^{b} \pm 0.23$	$1.78^{b} \pm 0.04$
−Al−++B	$21.85^{a} \pm 0.72$	$372^{a} \pm 11.86$	$6.25^{a} \pm 0.27$	$2.76^{a} \pm 0.13$
+Al−B	$7.55^{d} \pm 0.17$	$196^{c} \pm 11.21$	$3.26^{c} \pm 0.11$	$1.26^{c} \pm 0.03$
+Al+++B	$15.46^{b} \pm 0.4$	$343^{ab} \pm 13.46$	$5.33^{a} \pm 0.14$	$2.61^{a} \pm 0.11$
B	**	**	**	**
Al	**	**	**	**
B × Al	**	**	ns	*

Note: *−Al-B (2.5 µM B with 300 µM Al), µµ++Al-B (2.5 µM B and 300 µM Al), µ−Al+B (25 µM B with 0 µM Al as control) and, + ++Al+B (25 µM B and 300 µM Al). Different letters (a, b, c, d) in a column indicate significant differences among 4 treatments at 95% probability level with ± S.E; *, ** indicates significance differences at the $p < 0.05$ and $p < 0.01$ respectively; ns indicates non-significance.

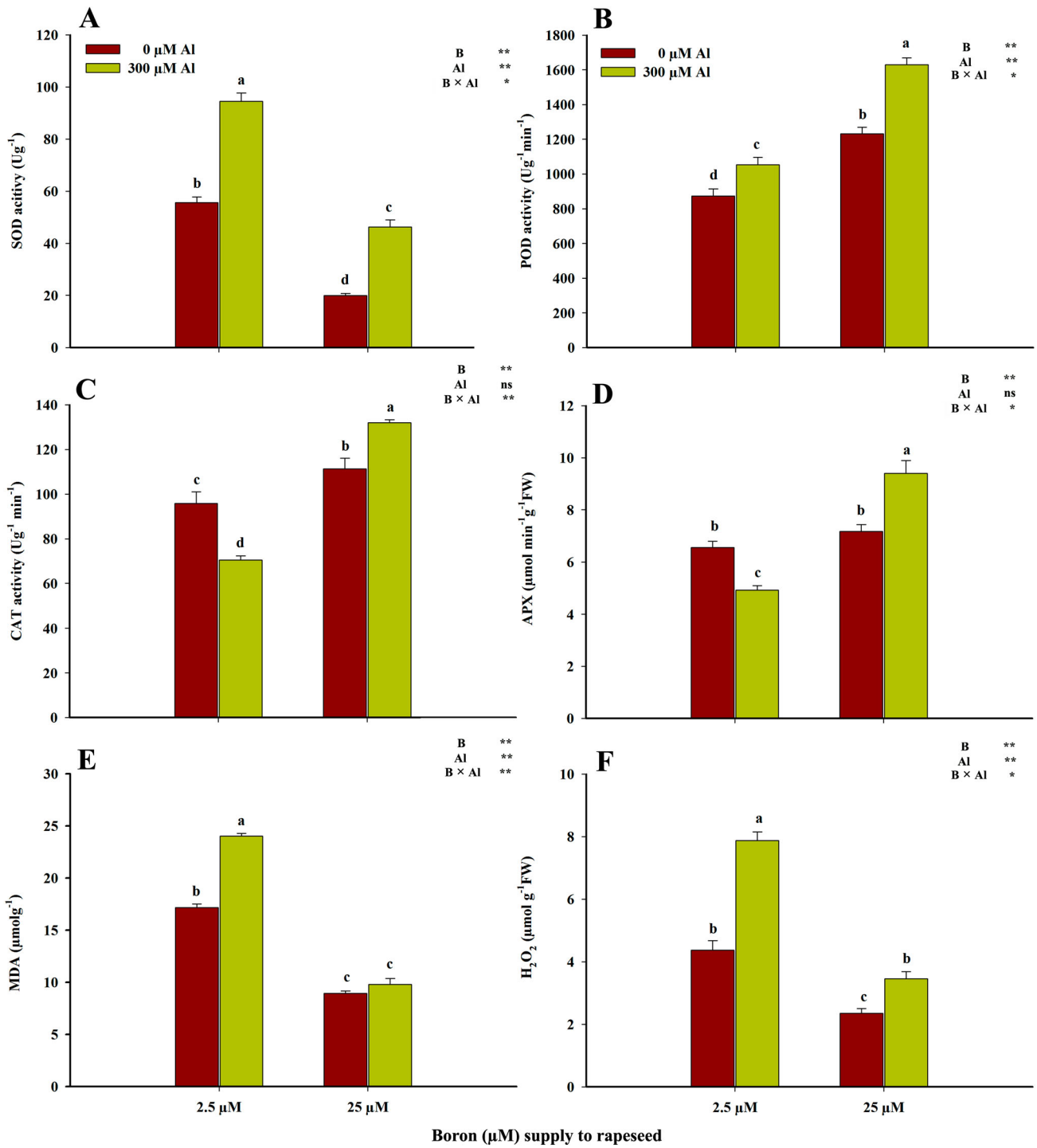

Figure 3. Antioxidant enzyme activities, MDA and H_2O_2 contents. (a) SOD (superoxide dismutase), (b) POD (peroxidase), (c) CAT (catalase), (d) APX (ascorbate peroxidase), (e) hydrogen peroxides (H_2O_2) and (f) MDA contents (malondialdehyde) in leaves of rapeseed subjected to different B and Al treatments i.e (−B 2.5 µM and ++B 25 µM, −Al 0 µM and ++Al 300 µM). Different letters (a, b, c, d) above column indicate significant differences among 4 treatments at $p < 0.05$.

90.76% respectively under Al toxicity. The Al under low B treatment resulted in a significantly higher oxidative stress and lipid peroxidation in terms of H_2O_2 and MDA content, respectively compared to the control treatment. Nevertheless, the 25 µM B supply has reduced production of H_2O_2 and the lipid oxidation in terms of MDA compared to low B treatment under Al toxicity (Figure 3).

Discussion

The root growth inhibition is the chief Al toxicity indication (Ryan et al. 1993). Extreme Al^{3+} toxicity restraints cell division as well as cell elongation (Čiamporová 2002). It has been reported that B supply could ameliorate Al-induced inhibition of root growth (Lenoble et al. 1996a, 1996b, Riaz et al. 2018b). Our study results showed that B could alleviate Al-induced inhibition of root growth in rapeseed. B-induced alleviation of Al toxicity on root growth and physiological parameters might be due to the enhanced response of antioxidant enzymes in response to elevated reactive oxygen species, thus reducing MDA (malondialdehyde) and H_2O_2 content.

Our results showed that under low B, the Al toxicity inhibited the root elongation and root morphological characteristics (Table 2). Results of the previous study on squash

plant showed that Al-toxicity or B-deficiency reduced the root elongation which could be an outcome of hindered ascorbate acid metabolism (Lukaszewski and Blevins 1996). Blevins (1987) proposed that Al toxicity might cause root inhibition by encouraging B deficiency due to similarities in symptoms of Al stressed and B-deficient plants. However, according to the results presented in our research showed that B concentration was found to be similar with or without Al (Yang et al. 2004; Stass et al. 2007; Jiang et al. 2009; Zhou et al. 2015). Hematoxylin results in the present study confirmed that roots were heavily stained with dense blue color under + Al–B treatment due to Al–hematoxylin complex as shown in Figure 2(c). It has been documented that B-deficiency increases the concentration of un-methylated pectin in the cell wall of roots, creating higher negative charges thus producing more favorable condition for Al binding (Stass et al. 2007; Li et al. 2017). Moreover, the role of B has been reported in the formation of the primary cell wall by cross-linking with pectic polypectic polysaccharide RG-II (rhamnogalacturonan II). This cross-linked RG-II gives rise to stable, complex cell wall with reduced pore spaces in the cell wall. So this complex network of cell wall hinders the entry of Al into the cells and prevents the direct contact of Al with cell organelles (O'Neill et al. 2004). Corrales et al. (2008) and Eticha et al. (2005) suggested that B reduces the level of negative charges, thus reducing Al binding to the cell wall. We found that Al concentration was lower under 25 μM B and our findings are in line with Yu et al. (2009) that B supply decreased the Al mobilization in plant parts as also revealed by Al-hematoxylin staining. Our results showed that Al toxicity severely hampered photosynthesis and photosynthetic pigments under low B, and adequate B supply alleviated Al-induced inhibition of photosynthesis and physiological characteristics (Table 3). This might be due to less uptake of Al into shoots, which have ultimately reduced chlorosis symptoms. Yu et al. (2009) reported that B alleviated the chlorosis of Al toxicity and prevented the decrease in chlorophyll concentration which was accompanied by lower Al level in the shoot. Likewise, Jiang et al. (2009) reported that B-induced alleviation of shoot growth, photosynthesis, and chlorophyll reduction might be as a result of less Al accumulation in the shoots.

The Al even at a low level could result in ROS production and ultimately damages to cell integrity (Yakimova et al. 2007). Oxidative stress due to Al is a decisive event leading to cell death (Yamamoto et al. 2003). Yamamoto et al. (1997) proposed that Al toxicity triggered lipid peroxidation of membranes and resulted in cell death. Corrales et al. (2008) reported that B is needed for plants to protect roots from oxidative damage under Al stress by improving defense system. The plants have evolved defense system consisting of both enzymatic as well as non-enzymatic components in order to better handle this elevated oxidative stress (Hussain et al. 2016). In the cascade of the enzymatic system, SOD is the first enzyme that transforms damaging free radicals to H_2O_2 and O_2, (Noctor and Foyer 1998). The CAT is considered an important and key enzyme for eliminating H_2O_2 (Ghanati et al. 2005). It has been reported that B supply under Al stress could increase and stimulate antioxidant activities, thereby, alleviates the Al-induced inhibition of root elongation (Lukaszewski and Blevins 1996). Ruiz et al. (2006) reported that increased application of B stimulates GSH biosynthesis (antioxidant) in response to high Al concentration in the growth medium, the activation of the defense system could reduce oxidative stress, and reduce phytotoxicity induced by Al toxicity. We found that 25 μM B resulted in the higher activities of antioxidant enzymes (POD, CAT, and APX) to cut down membranes lipid peroxidation by lowering hydrogen peroxide (H_2O_2) contents produced by Al toxicity. Normally, MDA is considered a good marker of lipid peroxidation and shows the extent of oxidative damage (Lopez-Martinez et al. 2008). Results of our study showed that oxidative stress caused by Al toxicity was effectively decreased after adequate B and this could be the reason behind the better tolerance of plants under + Al + B treatment. So, B is not only an essential microelement for plant growth but also might be effective in the amelioration of Al-toxicity.

Conclusions

The present study showed that the Al toxicity resulted in severe inhibition of root morphology, and physiological characteristics in the rapeseed. However, B supply ameliorated Al-induced inhibition of root growth parameters and physiological characteristics by reducing Al mobilization in the plant parts, and enhancing antioxidant enzyme activities and reducing oxidative damage. Our findings showed that B may provide better conditions and healthy environment to rapeseed for growth and to combat Al toxicity by alleviating some of Al toxicity symptoms.

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