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Aluminum alleviates boron-deficiency induced growth impairment in tea plants

R. HAJIBOLAND[1,2]*, S. BAHRAMI-RAD[2], and S. BASTANI[2]

Center of Excellence for Biodiversity, University of Tabriz, 51666-14779 Tabriz, Iran[1]
Plant Science Department, University of Tabriz, 51666-14779 Tabriz, Iran[2]

Abstract

Interaction between aluminum (Al) and boron (B) in Al accumulator species has not been characterized so far. In this work, tea [*Camellia sinensis* (L.) O. Kuntze] plants were cultivated hydroponically and treated with adequate (control) or low B supply (–B) without or with 300 µM Al (–B+Al) for 14 weeks. Growth of B-deficient plants was completely resumed by Al supplementation or even surpassed control plants regarding shoot biomass. Net photosynthetic rate was negatively influenced by the low B supply, and the Al treatment increased it up to the level of the control plants that was reflected in the higher content of saccharides. The activity of ascorbate peroxidase (APX) in the younger leaves decreased at the low B supply accompanied with an increased H_2O_2 content. The Al treatment increased the APX activity up to the level of the control plants simultaneously with the reduction of H_2O_2. Activities of superoxide dismutase (SOD) and peroxidase (POD) increased in the low B plants and the Al treatment augmented this effect. The content of malondialdehyde (MDA) in the leaves increased by low B but declined upon the Al treatment. In the Al-treated plants, the activity of nitrate reductase (NR) and the content of free α-amino acids exceeded those of the control plants, and nitrite concentration diminished. The shoot and root B content of the B-deficient plants supplemented with Al was similar with the B-sufficient ones. The results demonstrate that the up-regulation of C and N metabolism, the activation of antioxidative defense, and the enhancement of B uptake and transport were mechanisms for growth amelioration of the B-deficient plants by Al supplementation in tea.

Additional key words: ascorbate peroxidase, boron uptake and translocation, carotenoids, chlorophyll, net photosynthetic rate, nitrate, nitrite, peroxidase, stomatal conductance, superoxide dismutase, transpiration rate.

Introduction

Boron is an essential element for higher plants. In addition to its role in the cell wall, substantial evidence suggests that B is involved at least indirectly in diverse metabolic processes in plants including metabolism of saccharides, RNA, phenolics, and lignin, as well as respiration and membrane integrity (Cakmak and Römheld 1997, Brown *et al.* 2002). With a long list of metabolic symptoms in B-deficient plants, it was suggested that B deficiency results in a 'cascade effect' due to disruption of critical cellular processes (Broadley *et al.* 2012).

Aluminum is an abundant element in the earth's crust, and under acidic conditions, Al^{3+} is the dominant ionic species in the soil solution which is readily taken up by plants and may cause toxicity in susceptible species (George *et al.* 2012). The first symptom of Al toxicity in plants is reduction of root growth *via* inhibition of both cell elongation and cell division (Kochian 1995, Poschenrieder *et al.* 2008, Horst *et al.* 2010).

There are evidences on the interaction between Al and B in plants. Aluminum inside the plant is likely to be in the form of $Al(OH)_3$ which is structurally similar to boric acid H_3BO_3 (Kochian 1995). Both Al (Horst *et al.* 2010) and B (O'Neill *et al.* 2001) are mainly involved in the cell wall structure, membrane function, and root growth. In addition, root symptoms characteristic of Al-stressed plants are similar with those of B-deficient plants (Kochian *et al.* 2004).

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Abbreviations: APX - ascorbate peroxidase; Car - carotenoids; Chl - chlorophyll; E - transpiration rate; g_s - stomatal conductance; MDA - malondialdehyde; NR - nitrate reductase; NiR - nitrite reductase; P_N - net photosynthetic rate; POD - peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.

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* Author for correspondence; fax: (+98) 411 3356027, e-mail: ehsan@tabrizu.ac.ir

Interactions between B and Al have been studied in some Al sensitive species. Alleviation of Al inhibition of root growth by supraoptimal B, and an exacerbating effect of B-deficiency on Al toxicity have been reported in several Al sensitive species (LeNoble *et al.* 1996, Yang *et al.* 2004, Stass *et al.* 2007, Corrales *et al.* 2008, Jiang *et al.* 2009, Yu *et al.* 2009). Studies for the mechanisms of these interactions have been mainly focused on root growth and cell wall properties in the elongation zone (LeNoble *et al.* 1996, Corrales *et al.* 2008, Jiang *et al.* 2009, Yu *et al.* 2009).

Despite a wide range of metabolic alterations in B-deficient plants, the interaction between Al and B at the level of metabolism has attracted much less attention. Studies on Al sensitive species revealed that an extra supply of B to Al-stressed plants increases leaf CO_2 assimilation rate, activity of Rubisco, leaf Chl content, and Chl fluorescence parameters (Jiang *et al.* 2009). In addition, an application of B in the presence of high Al concentrations stimulates biosyntheses of glutathione (Ruiz *et al.* 2006, Corrales *et al.* 2008) and ascorbate (Lukaszewski and Blevins 1996) demonstrating that B alleviates Al stress *via* the enhanced ability of antioxidative defense. However, mechanisms for the interaction between Al and B in Al accumulator species tea have not been studied.

Tea is an Al-accumulator and it is cultivated on acid soils in areas with high rainfall and so with a high probability of B deficiency due to soil leaching (Shorrocks 1997). Beneficial effects of Al on growth of tea plants have been frequently reported (Matsumoto *et al.* 1976, Konishi 1985, Hajiboland *et al.* 2013a). We have previously demonstrated that, in contrast to Al-sensitive species, Al at concentrations as high as 300 µM causes a significant stimulation of root growth and shoot height in tea seedlings (Hajiboland *et al.* 2013a). The growth stimulating effect of Al in tea plants has been attributed to the enhancement of photosynthesis, activation of antioxidative defense (Hajiboland *et al.* 2013a), as well as the mitigation of Fe toxicity (Hajiboland *et al.* 2013b). Tea is relatively tolerant to B deficiency. In contrast to susceptible species, the induction of B deficiency symptoms in tea is not possible without removing contaminations of nutrient solution by using special resins (Hajiboland *et al.* 2011). However, significant modifications in the C and N metabolisms, such as reduction of photosynthesis, increase in the activity of nitrate reductase, and accumulation of nitrite in the young leaves occur under B deficiency (Hajiboland *et al.* 2011).

The aim of this study was to investigate the interaction of Al and B in tea seedlings. Our working hypothesis was that Al supplementation may alleviate growth reduction and metabolic disturbances evoked by B deficiency. This approach may elucidate some differences between Al sensitive and tolerant species.

Materials and methods

Hulled seeds of tea (*Camellia sinensis* (*L.*) O. Kuntze) were surface-sterilized with 1 % (m/v) hypo-chlorite and germinated on *Perlite* moistened with distilled water in the dark till emergence of primary leaves. One-month-old seedlings were transferred to 2 dm^3 plastic beakers containing a continuously aerated nutrient solution (pH 4.2) (Hajiboland *et al.* 2011). Boron treatments consisted of adequate boron supply (46 µM H_3BO_3; +B) and boron deficiency (without addition of B; –B). In order to minimize the contamination of the nutrient solution with B in the –B treatment, 1 g dm^{-3} washed B-specific resin (*Amberlite IRA 743, Fluka,* Buchs, Switzerland) packed in small textile bags was immersed in the nutrient solution. Two weeks after starting the B treatments, $AlCl_3$ at final concentration of 300 µM was applied for B-deficient plants (–B+Al). The nutrient solutions and the resin bags were replaced every week and pH was adjusted daily. Plants were grown under controlled environmental conditions with day/night temperatures of 25/18 °C, a relative humidity of 70/80 %, a 14-h photoperiod, and a photon flux density (PPFD) of about 400 µmol m^{-2} s^{-1}.

Ten weeks after starting the different B treatments (8 weeks after the Al treatments and 14 weeks after sowing), the plants were harvested. After the separation of shoots and roots, fresh masses, and after drying at 70 °C for 2 d dry masses, were determined. Oven-dried samples were transferred to porcelain crucibles and dry-ashed with 10 mg of $Ca(OH)_2$ at 550 °C for 5 h, resolved in 0.5 M HCl and made up to a volume of 25 cm^3 by double-distilled water. B and Al were determined using the *Azomethine-H* (*Sigma,* St. Louis, USA) method (Lohse 1982) and atomic absorption spectroscopy (*AA6300, Shimadzu*, Kyoto, Japan), respectively. Before harvest, leaf gas exchange parameters including net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were determined in attached leaves with a calibrated portable gas exchange system (*LCA-4, ADC Bioscientific,* Great Amwell, UK) between 10:00 and 13:00 at a PPFD of 400 µmol m^{-2} s^{-1} and a CO_2 concentration of 350 µmol mol^{-1}.

Biochemical analyses were carried out on the 2nd fully-expanded leaves form the top, the 3rd leaves from the bottom, and roots according to optimized protocols described previously (Hajiboland *et al.* 2011, 2013a). The content of Chl *a*, Chl *b*, and Car were determined spectrophotometrically (*Specord 200, Analytik Jena*, Jena, Germany) after leaf extraction in cold acetone (100 %) in the dark at 4 ºC for 24 h. Determination of anthocyanins was performed using the pH differential method (at pH 1 and pH 4.5) in pure methanol and 37 % HCl (98:2, m/v) and the content was expressed as mg(cyanidine-3-glucoside) g^{-1}(f.m.). The content of total flavonoids was determined in the methanol extract of leaves using quercetin (*Sigma*) as standard (Hajiboland *et al.* 2013a). For determination of saccharides, leaf tissues were homogenized in a 100 mM phosphate buffer (pH 7.5) at 4 ºC. After centrifugation at 12 000 *g* for 15 min, supernatant was used for determination of total soluble sugars, whereas the pellet was kept for starch analysis (Hajiboland *et al.* 2013a).

For assay of enzyme activities, fresh samples were immediately frozen and stored in liquid nitrogen. In brief, the activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured by determining ascorbic acid oxidation at 25 ºC (for more detail, see Hajiboland *et al.* 2013a). The peroxidase (POD, EC 1.11.1.7) activity was assayed using guaiacol (*Merck,* Darmstadt, Germany) as substrate (Hajiboland *et al.* 2013a). The total superoxide dismutase (SOD, EC 1.15.1.1) activity was determined using monoformazan formation and one unit was defined as the amount of enzyme required to induce a 50 % inhibition of reaction, compared with control samples without enzyme aliquot (Hajiboland *et al.* 2013a). Lipid peroxidation was estimated at 532 nm according to the amount of enzyme required to induce a 50 % inhibition of malondialdehyde (MDA) formation, compared with control samples without the enzyme aliquot (Hajiboland *et al.* 2013a) in a reaction mixture containing thiobarbituric acid (*Sigma*). The content of H_2O_2 was determined using potassium titanium-oxalate and absorbance was read at 508 nm (Hajiboland *et al.* 2013a).

The activity of nitrate reductase (NR, E.C. 1.6.6.1) was determined after vacuum-infiltration of leaf blades and root samples. Resulting nitrite was determined spectrophotometrically at 540 nm in a reaction mixture containing sulfanilamide and naphthylethylenediamine dihydrochloride (*N-NEDA, Sigma*) with $NaNO_2$ as standard (Hajiboland *et al.* 2011). A nitrate content was determined at 420 nm by formation of a nitro derivative of salicylic acid using KNO_3 as standard (Hajiboland *et al.* 2011). Soluble proteins were determined using a commercial reagent (*Bradford reagent, Sigma*) and bovine serum albumin *(Merck,* Darmstadt, Germany) as standard. The content of total free α-amino acids was assayed using the ninhydrin colorimetric method with glycine (*Merck,* Darmstadt, Germany) as standard (Hajiboland *et al.* 2011).

The experiment was undertaken in a complete randomized block design with four independent replications. Statistical analyses were carried out using the *Sigma Stat 3.5* software (*Systat Software Inc.,* San José, CA, USA) with theTukey's test ($\alpha = 0.05$).

Results

Plant dry masses decreased under B-deficiency up to 47 and 54 % in the shoots and roots, respectively. The treatment of the B-deficient plants with Al resumed the root dry mass completely. The dry mass of the shoots in the –B+Al plants was even higher than that of the control plants (Table 1).

Table 1. The dry masses of shoots and roots [g plant^{-1}] in tea plants grown under control and B deficiency (-B) conditions without or with Al (300 µM) supplementation (–B+Al) for 14 weeks. Data represent means ± SD from four independent replications. Data of each row indicated with the same letter do not differ significantly ($P > 0.05$).

	Control	–B	–B+Al
Shoot d.m.	1.70 ± 0.09^b	0.90 ± 0.06^c	1.90 ± 0.04^a
Root d.m.	0.52 ± 0.06^a	0.24 ± 0.01^b	0.56 ± 0.07^a

In the control plants, the older leaves were more dark green and had more Chl than the younger leaves. The content of Chl *a*, Chl *b*, and Car were not affected by the low B supply in the younger leaves, whereas a slight or even significant reduction of these pigments was observed in the older leaves (Table 2). Similarly, the leaf anthocyanins content declined at –B in the older leaves, but this reduction was not significant in the younger leaves. The content of flavonoids was not affected by the B-deficiency. The aluminum treatment of the B-deficient plants resulted in an increase of Chl *a*, Chl *b*, and Car in both the younger and older leaves, whereas the leaf anthocyanins content was not influenced, and the flavonoids content decreased (Table 2). P_N was negatively influenced by the low B supply especially in the younger leaves. This reduction was accompanied by lower g_s that resulted in lower E (Table 2). The Al treatment increased P_N of the B-deficient plants up to the level of the B-sufficient ones. A similar trend was observed for E and g_s (Table 2).

In the younger leaves, the content of soluble sugars was significantly lower in the –B plants but increased in the –B+Al ones. The starch content, however, increased in the –B plants and a further rise was observed upon the Al treatment (Table 2). In the older leaves, the soluble sugar content decreased slightly in the –B plants, but in contrast to the younger leaves, the Al treatment rather decreased the soluble sugar content in the older leaves. In contrast to the young leaves, the starch content of the older leaves was not significantly influenced by the different treatments (Table 2).

In the younger leaves, the activity of APX decreased at the low B treatment was accompanied with a higher H_2O_2 content. The Al treatment of the low B plants increased the APX activity up to the level of the control plants together with the reduction of H_2O_2 content. In contrast to APX, the activities of SOD and POD increased significantly under B deficiency and the Al treatment further increased them (Table 3). In the older leaves, the activities of all tested antioxidant enzymes

Table 2. The content of Chl *a*, Chl *b*, Car, anthocyanins, and flavonoids [mg g^{-1}(f.m.)], gas exchange parameters P_N [µmol (CO_2) m^{-2} s^{-2}], E [mmol (H_2O) m^{-2} s^{-2}], and g_s [mol (H_2O) m^{-2} s^{-2}], and the content of total soluble sugars [mg (eq. glucose) g^{-1}(f.m.)] and starch [mg g^{-1}(f.m.)] in younger and older leaves of tea plants grown under control conditions and B deficiency (−B) or with Al supplementation (–B+Al) for 14 weeks. Data represent means ± SD from four independent replications. Data of each row indicated with the same letter do not differ significantly ($P > 0.05$).

Treatments	Younger leaves			Older leaves		
	control	–B	–B+Al	control	–B	–B+Al
Chl *a*	0.50 ± 0.04^{b}	0.56 ± 0.05^{b}	1.01 ± 0.04^{a}	1.71 ± 0.14^{c}	1.42 ± 0.02^{b}	1.98 ± 0.08^{a}
Chl *b*	0.16 ± 0.04^{b}	0.14 ± 0.02^{b}	0.30 ± 0.03^{a}	0.47 ± 0.07^{ab}	0.42 ± 0.03^{b}	0.58 ± 0.05^{a}
CAR	0.17 ± 0.02^{b}	0.15 ± 0.00^{b}	0.31 ± 0.02^{a}	0.43 ± 0.03^{a}	0.35 ± 0.00^{b}	0.43 ± 0.02^{a}
Anthocyanins	25.9 ± 3.2^{a}	21.2 ± 2.6^{ab}	17.8 ± 0.9^{b}	26.0 ± 0.9^{a}	20.9 ± 0.3^{b}	25.9 ± 3.6^{ab}
Flavonoids	0.55 ± 0.07^{a}	0.65 ± 0.07^{a}	0.42 ± 0.05^{b}	0.78 ± 0.08^{a}	0.89 ± 0.10^{a}	0.37 ± 0.04^{b}
P_N	3.31 ± 0.27^{a}	2.36 ± 0.20^{b}	3.06 ± 0.36^{ab}	1.32 ± 0.06^{a}	1.10 ± 0.05^{a}	1.14 ± 0.03^{a}
E	1.04 ± 0.15^{a}	0.53 ± 0.24^{b}	0.72 ± 0.04^{ab}	0.33 ± 0.06^{a}	0.36 ± 0.11^{a}	0.25 ± 0.03^{a}
g_s	0.14 ± 0.04^{a}	0.03 ± 0.00^{b}	0.08 ± 0.03^{b}	0.04 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}
Soluble sugars	89.6 ± 11.0^{a}	65.0 ± 8.0^{b}	92.0 ± 13.0^{a}	102.0 ± 19.0^{a}	79.0 ± 8.0^{ab}	59.0 ± 6.0^{b}
Starch	5.2 ± 0.37^{c}	7.2 ± 0.31^{b}	11.9 ± 0.22^{a}	5.4 ± 0.27^{a}	5.3 ± 0.18^{a}	4.1 ± 0.24^{a}

Table 3. The activities of APX [µmol(ascorbate) mg^{-1}(protein) min^{-1}], SOD [U mg^{-1}(protein)], and POD [µmol(tetraguaiacol) mg^{-1}(protein) min^{-1}], and the content of H_2O_2 [µmol g^{-1}(f.m.)] and MDA [nmol g^{-1}(f.m.)] in younger and older leaves and roots of tea plants grown under B deficiency (−B) or with Al supplementation (–B+Al) for 14 weeks. Data represent means ± SD from four independent replications. Data of each column within each plant part indicated with the same letter do not differ significantly ($P > 0.05$).

Organs	Treatments	APX	SOD	POD	H_2O_2	MDA
Younger leaves	control	79 ± 12^{a}	1.32 ± 0.31^{c}	9.2 ± 0.11^{c}	23 ± 1.9^{c}	31 ± 8^{b}
	–B	50 ± 5^{b}	3.47 ± 0.97^{b}	19.1 ± 0.27^{b}	48 ± 3.2^{a}	59 ± 9^{a}
	–B+Al	76 ± 8^{a}	4.76 ± 0.43^{a}	25.3 ± 1.61^{a}	31 ± 4.7^{b}	19 ± 4^{b}
Older leaves	control	88 ± 22^{b}	1.58 ± 0.46^{b}	19.7 ± 2.24^{b}	43 ± 4.7^{b}	36 ± 6^{b}
	–B	124 ± 5^{a}	5.33 ± 0.85^{a}	23.8 ± 3.40^{b}	53 ± 4.3^{a}	54 ± 11^{a}
	–B+Al	91 ± 13^{b}	5.87 ± 0.11^{a}	34.5 ± 7.78^{a}	42 ± 3.8^{b}	35 ± 7^{b}
Roots	control	70 ± 4^{b}	0.84 ± 0.36^{c}	69.8 ± 16.7^{b}	36 ± 3.1^{a}	43 ± 12^{a}
	–B	87 ± 5^{a}	2.08 ± 0.59^{b}	176.2 ± 11.5^{a}	29 ± 1.3^{b}	38 ± 9^{a}
	–B+Al	79 ± 3^{a}	4.91 ± 0.05^{a}	90.3 ± 3.01^{b}	31 ± 2.8^{b}	41 ± 8^{a}

Table 4. The activity of NR [µmol(nitrite) g^{-1}(f.m.) min^{-1}], the content of nitrate [µmol g^{-1}(f.m.)], nitrite [nmol g^{-1}(f.m.)], total free α-amino acids [µmol g^{-1}(f.m.)], and soluble proteins [mg g^{-1}(f.m.)] in younger and older leaves and roots of tea plants grown under B deficiency (−B) or with Al supplementation (–B+Al) for 14 weeks. Data are means ± SD from four independent replications. Data of each column within each plant part indicated with the same letter do not differ significantly ($P > 0.05$).

Organs	Treatments	NR activity	Nitrate	Nitrite	Amino acids	Proteins
Younger leaves	control	3.30 ± 0.22^{c}	132 ± 7^{a}	69 ± 5^{b}	4.9 ± 0.8^{b}	2.1 ± 0.25^{a}
	–B	4.13 ± 0.48^{b}	119 ± 6^{b}	119 ± 8^{a}	4.8 ± 0.5^{b}	2.2 ± 0.51^{a}
	–B+Al	5.47 ± 0.11^{a}	117 ± 3^{b}	76 ± 3^{b}	8.3 ± 0.9^{a}	2.1 ± 0.98^{a}
Older leaves	control	3.91 ± 0.32^{b}	148 ± 6^{a}	99 ± 10^{a}	6.5 ± 0.9^{b}	2.0 ± 0.31^{a}
	–B	2.45 ± 0.25^{c}	130 ± 9^{a}	91 ± 9^{a}	6.8 ± 0.4^{b}	1.9 ± 0.41^{a}
	–B+Al	2.45 ± 0.25^{c}	146 ± 26^{a}	109 ± 18^{a}	11.2 ± 1.8^{a}	1.8 ± 0.81^{a}
Roots	control	7.85 ± 0.37^{b}	11 ± 4^{b}	93 ± 7^{a}	8.9 ± 1.3^{c}	1.1 ± 0.27^{a}
	–B	6.69 ± 0.45^{c}	23 ± 5^{a}	101 ± 7^{a}	14.1 ± 2.9^{b}	1.3 ± 0.41^{a}
	–B+Al	11.8 ± 0.48^{a}	16 ± 2^{b}	107 ± 9^{a}	21.2 ± 1.8^{a}	0.9 ± 0.36^{a}

increased under low B, and the Al treatment either decreased it (APX), did not affect (SOD), or increased it significantly (POD). Changes in the H_2O_2 content was similar as in the younger leaves but less pronounced (Table 3). In the roots, the activities of all tested antioxidant enzymes were higher under both the treatments compared with the control plants. The content of H_2O_2 was lower in the B-deficient plants, the opposite of the pattern observed in the leaves. But as expected, it was negatively correlated with the changes in the activity of APX (Table 3). The content of MDA in the leaves increased with low B but declined upon the Al treatment. In the roots, in contrast, the MDA content did not change significantly by the applied treatments (Table 3).

In the younger leaves, the activity of NR was higher in the –B plants and especially in the –B+Al plants. These changes were reflected in the significantly lower nitrate content in the younger leaves under both the treatments. The nitrite content, in contrast, was higher in the –B plants and diminished by Al supplementation to levels in the control plants (Table 4). In the older leaves and in the roots, B deficiency decreased the NR activity, whereas Al treatment increased it. The nitrate content in the roots reflected the changes in the NR activity, *i.e.*, it increased in the –B but was reduced in –B+Al ones. In the older leaves, however, the nitrate content remained almost constant. The nitrite content in the older leaves and in the roots was not significantly affected by the –B or –B+Al treatments. The content of amino acids did not change significantly in the leaves but increased in the roots under B deficiency. The Al treatment increased the content of free α-amino acids in both the leaves and the roots. The content of soluble proteins, however, remained almost unchanged under both the treatments (Table 4).

The shoot and root B content, as expected, was significantly lower in the B-deficient plants. Interestingly, in the Al-supplemented plants, the B content was similar with the B content in the B-sufficient plants in both the shoots and the roots (Table 5). The Al content was higher in the Al-supplemented plants, but considerable Al was also found in the control plants without Al supply. The B-deficient plants had lower Al in both the shoots and the roots (Table 5).

Table 5. The content of B and Al [μg plant^{-1}] in leaves and roots of tea plants grown under B deficiency (−B) or with Al supplementation (–B+Al) for 14 weeks. Data represent means ± SD from four independent replications. Data of each row within each plant part indicated with the same letter do not differ significantly ($P > 0.05$).

Treatments	Leaves control	–B	–B+Al	Roots control	–B	–B+Al
B content	190 ± 28^{a}	70 ± 12^{b}	182 ± 19^{a}	36 ± 8^{a}	13 ± 4^{b}	39 ± 11^{a}
Al content	507 ± 91^{b}	147 ± 25^{c}	1221 ± 119^{a}	150 ± 20^{b}	55 ± 17^{c}	260 ± 24^{a}

Discussion

A significant growth depression observed in both the shoots and the roots under B deficiency could be primarily attributed to the reduction of photosynthesis. A reduction of nitrate assimilation in the older leaves and the roots that could lead to lower protein synthesis was likely another mechanism. Because of a marked biomass reduction, the protein content per fresh mass unit was not influenced by low B, but considering the total protein in plant, an inhibition of protein synthesis could be demonstrated. A reduction of NR activity due to low B has been reported in tobacco (Camacho-Cristóbal and González-Fontes 1999). An unexpected increase in the NR activity in the younger leaves in the B-deficient plants could be considered as metabolic acclimation because in contrast to the older leaves, they have been developed under B deficiency. We have also reported an acclimation to B deficiency in young leaves of drought-stressed tea plants (Hajiboland and Bastani 2012).

Another reason for growth impairment was an imbalance between production and scavenging ROS that was reflected in a higher MDA content in the leaves. The antioxidative defense system is comprised of several enzymes, such as SOD that is responsible for scavenging superoxide radicals and for production of H_2O_2 which is further metabolized by APX and POD. Under B deficiency, the higher SOD activity accompanied with the lower APX activity in the younger leaves resulted in a two-fold increase in the H_2O_2 content in the B-deficient plants. Despite an increase in the POD activity, it was apparently not sufficiently efficient for H_2O_2 scavenging in the younger leaves. In addition, elevated activities of antioxidative enzymes in all plant organs were not sufficient for detoxification of ROS as could be judged by increased membrane damage in the B-deficient plants (Table 3). These results may indicate higher rates of ROS generation than scavenging under B deficiency.

The nitrite content in the younger leaves was significantly higher under B deficiency. Nitrite is toxic to the cell because it forms diazo-compounds with amino groups of nucleobases and leads to mutations. Under normal metabolic conditions, the very efficient reduction of nitrite by nitrite reductase (NiR) prevents nitrite accumulating in the cell (Heldt 2005). Thus, disruption in this coupling because of lower NiR activity and/or lack of sufficient reducing equivalents might be the reason for the nitrite accumulation in the B-deficient plants. However, the relevance of the nitrite accumulation in the growth reduction under B deficiency conditions, and triggering B-induced metabolic disturbances needs to be elucidated. B deficiency influences many processes in plants rather indirectly likely *via* evoking some cascade effects that may influence, in turn, down-stream metabolic pathways (Broadley *et al.* 2012).

The ameliorating effect of Al supplementation on the B-deficient tea plants that is documented in this work may be related to the interactions between Al and B in their effects on cell wall properties or metabolism of the whole plant. In this work, we studied the latter mechanism with an emphasis on the antioxidant defense system as well as C and N metabolisms.

The most important effect of Al in tea plants under optimum growth conditions is an enhancement of CO_2 assimilation that causes, in turn, an increase in the soluble sugar content of leaves (Hajiboland *et al.* 2013a). In this work, P_N of the –B plants was partially resumed by Al supplementation. However, g_s was not significantly increased by Al suggesting an involvement of non-stomatal factors in elevating P_N. An improvement of N assimilation and protein synthesis (see below) is likely the most important mechanism for the elevated photosynthetic C metabolism in the Al-supplemented plants. To achieve higher rates of CO_2 assimilation, large numbers of photosynthetic components are necessary which could be provided by higher amounts of N invested in the photosynthetic machinery (Lawlor 2002). The higher content of leaf Chl and Car might be also partly responsible for up-regulation of P_N by Al in the younger leaves.

The activity of NR increased upon the Al treatment in both the leaves and the roots, it even exceeded that of the control plants. The elevated NR activity was accompanied with the higher amino acid content in the Al-treated plants. Unexpectedly, an Al-mediated increase in the NR activity was not associated with the lower nitrate content in the leaves. It is likely because of a higher uptake and root-shoot translocation of nitrate due to the Al treatment, the latter was probably the reason for nitrate depletion in the roots. In soil grown tea plants, Al increases the uptake of N, P, and K (Osaki *et al.* 1997) that has been considered one of the mechanisms for improved growth of tea plants.

A reduction of nitrite content in the younger leaves to the level of the control plants by Al supplementation likely indicates a re-establishment of coupling between NR and NiR activities by Al. It was probably because of an improvement in the electron transport rate in chloroplasts providing sufficient reducing equivalents for NiR activity. The content of NADH plays a critical role in nitrate and ammonia assimilations (Dutilleul *et al.* 2005). We previously observedslight rises of excitation-capture efficiency of open photosystem (PS) II centers, quantum yield of PS II, and linear electron-transport rate in leaves of Al treated tea plants (Hajiboland *et al.* 2013a).

The Al treatment stimulated the activities of antioxidative enzymes and decreased the H_2O_2 and MDA content significantly. The concentration of superoxide radical was not determined in this work. However, a positive correlation (r = 0.69, P < 0.05) between the content of H_2O_2 and MDA likely indicates that activities of H_2O_2 detoxifying enzymes, particularly APX, were crucial for antioxidative defense capacity in the tea plants under B-deficiency and Al supplementation. The activation of antioxidative defense by Al has previously been reported for *Plantago* species (Martins *et al.* 2013) and tea plants grown under optimum nutritional and environmental conditions (Hajiboland *et al.* 2013a). This mechanism may be also effective for plants grown under the excess of Fe (Hajiboland *et al.* 2013b) that is common in acid soils of tea plantations especially in areas affected by heavy rainfalls combined with poor soil drainage (Rao *et al.* 2007).

We observed a significant effect of Al on the uptake and translocation of B and *vice versa*. Al supplementation caused a significant increase of the root and shoot B content at the low B supply indicating the effect of Al on both root uptake and root-shoot translocation of B. The higher shoot B content may be partly attributable to the enhanced transpiration stream in the Al-supplemented plants. The transport of B into shoots is mediated mainly by transpiration stream and shoot organs with a higher transpiration rate have a higher B content (Hu and Brown 1997). However, regarding only a slight increase of transpiration upon the Al treatment, other mechanisms should be also involved in the Al-mediated increase of B transport. These mechanisms should explain not only a higher root-shoot B transport but also a greater root B uptake. A greater root surface that may be important for soil-grown plants, and lower hydrophobic wall barriers due to a diminished lignin synthesis (Hajiboland *et al.* 2013a) that, in turn, facilitates radial water flow, could be involved in the Al-mediated increase in B uptake and transport in this work. These mechanisms may cause a higher uptake and transport of not only B but also other nutrient ions, such as nitrate, as discussed above. Increase in nutrient uptake by Al in tea plants under field conditions has been well documented and considered as one of the mechanisms for Al-improved growth in tea plants (Osaki *et al.* 1997). Detailed works are needed for study of the mechanisms for Al effect on nutrient uptake in general and on B uptake in soil grown plants in particular.

The plants without an additional Al supply showed a considerable Al accumulation [~250 $\mu g\ g^{-1}$(d.m.)] in the leaves and the roots (data not shown). This high Al content could be derived from the nutrient solution that might be extracted by tea roots with a higher efficiency. The seeds were the other possible source of Al. The same high Al content in control plants have been reported in our previous works (Hajiboland *et al.* 2013a,b) where Al was determined by different methods.

In Al sensitive species, Al content is high in low B plants and it decreases with increasing B supply (Wojcik 2003, Jiang *et al.* 2009, Yu *et al.* 2009) that is likely the result of an increase in potential binding sites for Al in B-deficient plants (Stass *et al.* 2007). In contrast, the B deficiency reduced the root and shoot Al content in the tea plants. It could be concluded that in the tea plants, the effect of B deficiency on Al uptake is comparable with its effect on uptake of other plant nutrients. Reduction in the uptake of nutrients in B-deficient plants has been documented (Wojcik 2003, Broadley *et al.* 2012). B is involved in the regulation of membrane integrity and functioning, stomatal opening, as well as activity of H^+ATPase, and thus in the membrane potential and ion transport processes (Broadley *et al.* 2012). Accordingly, the reduction of Al in tea might be the result of a general diminishment of ion transport across membranes and a lower root-shoot translocation due to a lower transpiration flow in the B-deficient plants.

In conclusion, our results demonstrate that Al supplementation ameliorated the growth reduction of the B-deficient plants *via* several mechanisms. In addition to activating antioxidative defense and the enhancement of B uptake and transport, the up-regulation of C and N metabolisms was likely of great importance for positive responses of the B-deficient tea plants to Al. Plant growth and metabolism are highly dependent on the interaction between C and N metabolisms (Lawlor 2002). The Al-supplemented plants had not only the higher P_N but also assimilated more N. CO_2 assimilation rate is related to leaf N supply because N determines the synthesis of proteins and ultimately of all photosynthetic components. In turn, the assimilation of nitrate requires reducing equivalents, ATP, and C skeletons (Heldt 2005). The beneficial effect of Se on plant growth has also been attributed to the activation of CO_2 and nitrate assimilations (Hajiboland and Sadeghzadeh 2014).

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