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Photosynthesis, Nitrogen Metabolism and Antioxidant Defense System in B-Deficient tea (*Camellia sinensis* (L.) O. Kuntze) Plants

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Abstract

Response of tea plants to B deficiency was studied in hydroponic medium under environmentally controlled conditions. Plants height, number of leaves and dry matter production of shoot and root were significantly decreased by B deficiency. Concentration of chlorophyll, carotenoids, anthocyanins and flavonoids was not affected by B deficiency in the young leaf, while a significant reduction of Chl a/b ratio and increase of carotenoids concentration was detected in the old leaf. Among leaf chlorophyll fluorescence parameters, only non-photochemical quenching (*qN*) increased in parallel to increase of carotenoids in the old leaves of B-deficient plants. Net assimilation (*A*) and transpiration (*E*) rate and stomatal conductance (g_s) were decreased significantly under B deficiency conditions only in the young leaf. Activity of nitrate reductase increased in the young and decreased in the old leaf slightly, but was not influenced by B deficiency in the roots. Nitrate concentration diminished while nitrite concentration increased in the young leaf under B starvation. Data of antioxidant enzymes activity implied that ascorbate peroxidase was likely important for the protection of old leaf and peroxidase for roots, while superoxide dismutade contributed to the protection of all three organs against oxidative stress provoked by B deficiency conditions. H_2O_2 content of leaves and roots did not increase or even decreased under B deficiency conditions, that was associated with accumulation of proline. Our evidences suggest a protective role for proline against free radicals that may contribute significantly to the higher tolerance of tea plants to B deficiency.

Keywords: Chlorophyll fluorescence parameters; Critical B deficiency content; Gas exchange; Nitrate reductase activity; Proline

Introduction

Boron (B) is an essential micronutrient for vascular plants. However, the role of B in plants is still least understood [29]. Boron is directly or indirectly involves in many physiological and biochemical processes during

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plant growth, such as cell elongation and division, cell wall biosynthesis, membrane function, leaf photosynthesis and nitrogen metabolism [10].

Previous studies have shown that B deficiency affects photosynthesis directly or indirectly [46]. Decreased rate of CO_2 assimilation appears to result from reduced chlorophyll (Chl) content, photosynthetic electron transport rate and photophosphorylation [35] and is accompanied by structural and functional damage [15]. Despite of decreased photosynthesis, accumulation of starch and hexose occurs because growth is more affected than photosynthesis [46]. Since carbon metabolism is fundamental for plant growth, non-structural carbohydrates concentration and composition in plant tissues are important indicators for interpreting plant carbon status [46].

Plants require large amounts of nitrogen for the biosynthesis of amino acids and secondary metabolites. Nitrate reduction catalysed by nitrate reductase (NR), is the primary step in the nitrate assimilation process, and it is essential for the production of ammonium to be incorporated into carbon skeletons for amino acids biosynthesis. It was shown that B deficiency causes a drop in leaf NR activity and nitrate concentration of tobacco plants compared to control leaves [11]. Accordingly, study of carbon and nitrogen metabolism in B-deficient plants could elucidate physiological functions of B in plants.

Oxidative burst is a common response of plant cells to biotic and abiotic stresses [3]. The production of reactive oxygen species (ROS) was reported to occur in plants when they are challenged by pathogens and also when they are exposed to environmental stresses as well as nutritional imbalances such as ion toxicity and deficiency conditions. These radicals are very toxic to the membrane proteins and lipids which cause disintegration and destabilization of the cellular membranes and result in membrane leakage [3]. To minimize cellular damage caused by ROS, plants have evolved a scavenging system composed of antioxidant metabolites and enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT). Although there have been several reports investigating the antioxidant responses in leaves of plants treated with excess B [31], data relating to the effect of B deficiency are limited. It was reported that, low B decreased SOD, APX and CAT activities in soybean [25]. In sunflower, B deficiency increased APX activity but decreased CAT and did not affect SOD activity [10,14]. In citrus seedlings, B deficiency increased SOD and APX but reduced CAT activity [20]. In turnip plants, although activity of antioxidant defense enzymes was elevated under B deficiency, they did not provide sufficient protection from oxidative damage as judged by higher accumulation of malondialdehyde (MDA), H_2O_2 and tissue K^+ leakage compared with control plants [19].

Tea (*Camellia sinensis* (L.) O. Kuntze) is a native species of south China and cultivated in humid and subhumid of tropical, subtropical and temperate regions of the world usually on acid soils. In Iran tea is cultivated on acid soils in the north of the country. In spite of a large amount of works on the phytochemistry of tea leaves, research works on mineral nutrition of this plant are rare [38, 39]. Boron deficiency is generally related to high rainfall areas and acid soil conditions common in soils of tea plantations. Under acid soil conditions, B is more water-soluble and can therefore be leached below the root-zones of plants by rainfall or irrigations [42]. To our knowledge, there is hardly any information on the responses of tea plants to B deficiency.

The objective of this work was to evaluate the effects of B deficiency on growth, B concentration, gas exchange and nitrogen metabolism in tea plants. An attempt was made to study the antioxidant defense system in order to understand the mechanisms of B starvation effect on growth of tea plants.

Materials and Methods

Plants Culture and Treatments

Seeds of tea (*Camellia sinensis* (L.) O. Kuntze) plants were collected from the garden of Tea Research Station in Fuman (Guilan Province, Iran). Hulled seeds were surface-sterilized with 1% active hypochlorite and germinated on perlite in the dark and moistened by distilled water and saturated $CaSO_4$ every day. After emergence of the primary leaves, seedlings were transferred to the light. One month-old seedlings were transferred to the 2 L pots containing nutrient solution with the following composition (in mM): $(NH_4)_2SO4$ 0.71, NH_4NO_3 0.73, KH_2PO_4 0.1, K_2SO_4 0.46, $CaCl_2$ 0.5, $MgSO_4$ 0.41, Fe-EDTA 0.032, $CuSO_4$ 0.002, $MnSO_4$ 0.09, Na_2MoO_4 0.0026, $ZnSO_4$ 0.009 [16]. Three plants were cultivated in each pot, pH was adjusted to 4.2 and nutrient solution was replaced every 5 days. Boron treatments consisted of adequate (46 µM) supply (+B) and deficiency (–B) without B addition to the nutrient solution. In order to minimize the B contamination of nutrient solution in –B treatment, 1 g L^{-1} of washed B specific resin (Amberlite IRA 743, Fluka) [4] packed in small textile bags were kept immersed in the nutrient solution throughout the plants cultivation. Nutrient solution and resin bags were

replaced every one week. Plants were grown for 6 weeks under controlled environmental conditions with a temperature regime of 25/18 °C day/night, 14/10 h light/dark period, a relative humidity of 70/80% and at a photon flux density of about 400 µmol $m^{-2}s^{-1}$.

Plant Harvest and Analysis

Six weeks after treatment (10 weeks after sowing), plants were harvested. After drying at 70 °C for 2 days to determine dry weight, oven-dried samples were transferred to porcelain crucibles and dry-ashed with 10 mg $Ca(OH)_2$ at 550 °C for 5 h, resolved in 0.5 M HCl and made up to volume by double-distilled water. Boron was determined following the azomethine-H method as described by *Lohse* [26]. Before harvest, Chl fluorescence and gas exchange parameters were determined in the attached leaves.

Determination of Chlorophyll Fluorescence and Gas Exchange Parameters

Chlorophyll fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK) for both dark adapted and light adapted leaves. Measurements were carried out on the old and young, fully-expanded leaves. An average of 4 records from different parts of each individual leaf was considered for each replicates. Leaves were acclimated to dark for 30 min using leaf clips before measurements were taken. Initial (F_0), maximum (F_m), variable ($F_v=F_m-F_0$) fluorescence as well as maximum quantum yield of PSII (F_v/F_m) were recorded. Light adapted leaves (400 µmol $m^{-2}s^{-1}$) were used for measurement of initial (F_t) and maximum (F'_m) fluorescence. Calculations were made for F'_0 ($F'_0=F_0/[(F_v/F_m) +(F_0/F'_m)]$), effective quantum yield of PSII, Φ_{PSII} $[(F'_m-F_t)/F'_m]$, photochemical quenching, qP $[(F'_m-F_t)/(F'_m-F'_0)]$ and non-photochemical quenching, qN $(1-[(F'_m-F'_0)/(F_m-F_0)])$ [34].

CO_2 assimilation and transpiration rates were measured in parallel with Chl fluorescence measurements in the same leaf with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) between 10:00 and 13:00 at harvest. The measurements were conducted with photosynthetically active radiation (PAR) intensity at the leaf surface of 400 µmol m^{-2} s^{-1} measured by a quantum sensor attached to the leaf chamber of the gas exchange unit. The net photosynthesis rate by unit of leaf area (A, µmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and the stomatal conductance to water vapor (g_s, mol m^{-2} s^{-1}) were calculated using the values of CO_2 and humidity variation inside the chamber, both measured by the infrared gas analyzer of the portable photosynthesis system.

Determination of Chlorophyll, Carotenoids, Anthocyanins and Flavonoids

Leaf concentration of Chl a, b and carotenoids were determined after extraction of pigments in the cold acetone and allowing the samples to stand for 24 h in the dark at 4 °C [24]. Determination of anthocyanins was performed using a pH differential method at pH 1 and pH 4.5 in the methanol/HCl (98:2, v/v) extract [17]. Concentration of total anthocyanins was expressed as g of cyanidine-3-glucoside g^{-1} FW. Total flavonoids content was determined in the methanol extract of leaves. An aliquot of 1 mL of the solution containing 1 mg extracts in methanol was added to test tubes containing 0.1 mL of 10% $Al(NO_3)_3$, 0.1 mL of 1 M potassium acetate and 3.8 mL of methanol. After 40 min at room temperature, the absorbance was recorded using spectrophotometer at 415 nm. Quercetin was used as a standard [40].

Determination of Carbohydrates

Leaves were homogenized in 100 mM phosphate buffer (pH 7.5) at 4 °C, after centrifugation at 12000 *g* for 15 min, supernatant was used for determination of total soluble sugars whereas the pellets were kept for starch analysis [30].

Assay of Antioxidant Enzymes and Related Metabolites

For determination of the activity of antioxidant enzymes and concentration of related metabolites fresh samples were ground in liquid nitrogen and measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany). The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured by determining ascorbic acid oxidation at 25 °C [8]. Peroxidase (POD, EC 1.11.1.7) activity was assayed using the guaiacol test, the enzyme activity was calculated as enzyme protein required for the formation of 1 µmol tetraguaiacol min^{-1} [13]. Total superoxide dismutase (SOD, EC 1.15.1.1) activity was determined using monoformazan formation test [17]. One unit of SOD was defined as the amount of enzyme required to induce a 50% inhibition of NBT reduction as measured at 560 nm, compared with control samples without enzyme aliquot. Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a

reaction mixture containing thiobarbituric acid (Sigma) at 532 nm. MDA levels were calculated from a 1,1,3,3-tetraethoxypropane (Sigma) standard curve [8]. The concentration of H_2O_2 was determined using potassium titanium-oxalate at 508 nm [8]. Proline was extracted and determined by the method of *Bates et al.* [5]. Leaf tissues were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged at 3000 *g* for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined. Proline (Sigma) was used for production of a standard curve.

Determination of Soluble Protein and Total Free Amino Acids

Soluble proteins were determined as described by *Bradford* [7] using a commercial reagent (Sigma) and BSA (Merck) as standard. Content of total free α-amino acids was assayed using a ninhydrin colorimetric method [21]. Glycine was used for standard curve.

Nitrate Reductase Activity

In vivo nitrate reductase (NR, E.C. 1.6.6.1) activity was determined using the method described by *Jaworski* [22]. Leaf blades and root samples were cut into 5 mm sections and placed in incubation buffer (100 mg tissue for 10 ml of buffer) containing 25 mM K-phosphate buffer (pH 7.2), 25 mM KNO_3, and 1% Triton X-100. The samples were infiltrated using a vacuum (0.8 bar). After 1 (roots) or 5 (leaves) h, the vacuum was released and the samples were incubated at 30 °C in darkness for 1 h then placed in a boiling water-bath to stop the NR activity. The resulting nitrite concentration was determined using spectrophotometer at 540 nm in a reaction mixture containing 2 ml of extract, 2 ml of 1% (w/v) sulfanilamide in 1.5 M HCl, and 2 ml of 0.02% (w/v) N-NEDA (naphthylethylenediamine dihydrochloride) in 0.2 M HCl. Activity of NR was calculated from a standard curve established with $NaNO_2$ concentrations and expressed in produced mmol NO_2^- h^{-1} g^{-1} FW.

Nitrate and Nitrite Concentration

Nitrate was determined according to the procedure developed by *Cataldo et al.* [12]. Standard curve was created using KNO_3 in the range of 0-10 mM. Nitrite was extracted from fresh materials using 25 mM potassium phosphate buffer (pH 7.5). After centrifugation and proper dilution nitrite concentration was assayed after formation of a red-violet colored water soluble Azo dye as used for determination of nitrite produced by the activity of NR described above.

Experiment was undertaken in complete randomized block design with 4 replications. Statistical analyses were carried out using Sigma Stat (3.02) with Tukey test ($p<0.05$).

Results

Visual difference between B-sufficient and B-deficient plants was observed 30-35 days after starting treatment as reduced shoot height. Leaf symptoms were appeared only in the young leaves include reduced surface area, dark green color and rarely, curling of leaf lamina. Roots of B-deficient plants were poorly branched and tended to turn light purple. Boron-deficient plants have up to 40% lower shoot and root dry weight (DW) (Fig. 1). Number of leaves and height of shoot were also reduced by about 32% and 17%, respectively (data not shown). Boron concentration of the young and old leaves was significantly lower under B deficiency conditions, while in the roots only a slight reduction of B concentration was observed. Boron concentration of the old leaves was significantly higher

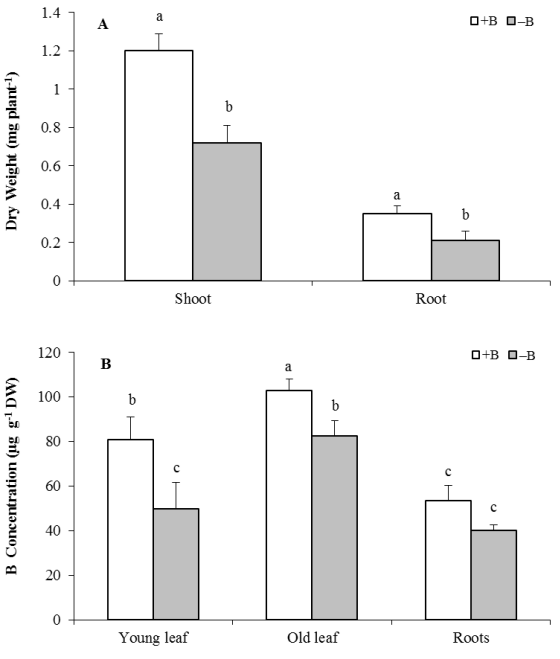

Figure 1. Dry weight (mg $plant^{-1}$) of shoot and root (A) and boron concentration (µg g^{-1} DW) (B) of three different fractions in B-sufficient (+B) and B-deficient (–B) tea (*Camellia sinensis* L.) plants. Bars indicated by the same letter are not significantly different ($P<0.05$).

than the young leaves and roots irrespective to the B supply level (Fig. 1).

Leaf concentration of Chl, carotenoids, anthocyanins and flavonoids was not affected significantly by B supply level in the young leaf. In the old leaf, however, leaf Chl a and b concentration decreased and increased by B deficiency respectively, leading to a significant reduction of Chl a/b ratio from 2.69 to 0.94 in this leaf. Carotenoid concentration of the old leaf was also increased significantly in B-deficient plants (Table 1).

Maximum quantum yield of PSII (F_v/F_m), excitation capture efficiency of open PSII (F'_v/F'_m), and photochemical quenching (q_P) were not influenced by B supply level either in the young or in the old leaves. Quantum yield of PSII (Φ_{PSII}) was lower in the young compared with the old leaf, but significant effect of B deficiency on Φ_{PSII} was not observed in the leaves with different age. Non- photochemical quenching (q_N) was significantly higher in the old leaf under B deficiency conditions (Table 1).

Net assimilation rate (A) was decreased significantly in the young leaf, however, this reduction was not significant in the old leaf. Similarly, transpiration rate and stomatal conductance were declined significantly only in the young leaf due to B deficiency (Fig. 2).

Soluble sugars content decreased in response to B deficiency conditions, the effect was significant only in the old leaf. In contrast to soluble sugars, starch content tended to increase in B deficient plants, however, difference between +B and –B plants was not significant (Fig. 3).

Boron supply level affected slightly the NR activity. In the young leaf, B deficiency caused a slight increase in NR activity, but in the old leaf the opposite was observed. The endogenous activity of NR in the roots was more than twice the activity observed in the leaves. Irrespective to the B nutritional status of plants, nitrate concentration was highest in the old leaf followed by the young leaf and roots. In contrast to the old leaf and roots, in the young leaf the amounts of nitrate and nitrite were influenced significantly by B deficiency. However, nitrate concentration diminished while the nitrite concentration increased in the young leaf of B starved plants. Only a slight reduction of total free amino acids and soluble proteins was observed in the leaves under B deficiency conditions. In the roots, in contrast, rather an increase of total amino acids was observed under B deficiency conditions (Table 2).

Activity of APX decreased under B deficiency conditions in the young leaf, increased significantly in the old leaf while was not affected in the roots. Activity of POD was slightly increased in the young and old leaves by B deficiency. However, the effect of deficiency conditions was much more pronounced in the roots with up to 182% rise in the activity in B-deficient plants. Superoxide dismutase (SOD) activity was significantly higher in all three examined organs under B deficiency conditions compared with control. Malondialdehyde (MDA) content of both young and old leaves was significantly higher in B-deficient plants in comparison to B-sufficient ones. In contrast, root MDA content was reduced in B-deficient plants. Hydrogen peroxide (H_2O_2) content of leaves and roots was either not influenced significantly or rather decreased by B deficiency. In contrast, proline content of all three tested organs increased under B deficiency conditions significantly (Table 3).

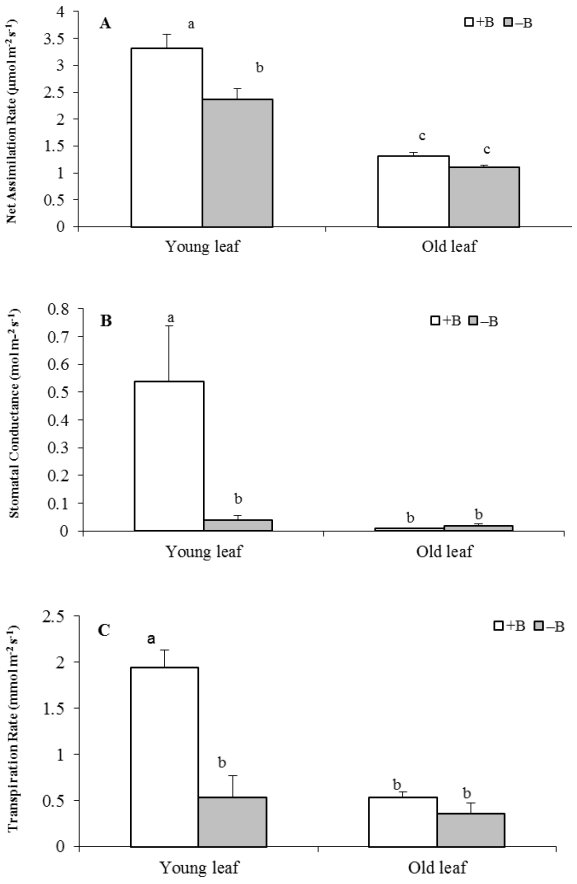

Figure 2. Gas exchange parameters including net photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}) (A), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) (B) and stomatal conductance to water vapor (g_s, mol m^{-2} s^{-1}) (B) in the young and old leaves of B-sufficient (+B) and B-deficient (–B) tea (*Camellia sinensis* L.) plants. Bars indicated by the same letter are not significantly different (P<0.05).

Table 1. Leaf concentration of chlorophyll (Chl) a, b and carotenoids (mg g^{-1} FW), anthocyanins (mg cyanidin-3-glucosid g^{-1} FW) and flavonoids (mg g^{-1} FW) and chlorophyll fluorescence parameters including F_v/F_m (photochemical efficiency of PSII), F'_v/F'_m (excitation capture efficiency of open PSII), Φ_{PSII} (quantum yield of PSII), q_P (photochemical quenching) and q_N (non-photochemical quenching) in the young and old leaves of B-sufficient (+B) and B-deficient (−B) tea (*Camellia sinensis* L.). Data in each column within each parameter followed by the same letter are not significantly different (P<0.05)

	Treatments	Chl a	Chl b	Carotenoids	Anthocyanins	Flavonoids
Young leaf	+B	1.06±0.13 ^{b}	0.41±0.06 ^{c}	86±10 ^{c}	25.9±2.3 ^{a}	0.55±0.07 ^{c}
	−B	1.15±0.27 ^{b}	0.49±0.09 ^{c}	100±18 ^{c}	21.6±3.2 ^{a}	0.65±0.07 ^{bc}
Old leaf	+B	3.52±0.34 ^{a}	1.28±0.13 ^{b}	254±19 ^{b}	29.6±6.2 ^{a}	0.83±0.13 ^{ab}
	−B	1.56±0.51 ^{b}	1.71±0.25 ^{a}	331±48 ^{a}	20.9±6.3 ^{a}	0.89±0.10 ^{a}
		F_v/F_m	F'_v/F'_m	Φ_{PSII}	q_P	q_N
Young leaf	+B	0.74±0.04 ^{a}	0.73±0.03 ^{a}	0.70±0.03 ^{b}	0.96±0.05 ^{a}	0.14±0.03 ^{ab}
	−B	0.79±0.01 ^{a}	0.77±0.02 ^{a}	0.71±0.01 ^{b}	0.93±0.02 ^{a}	0.13±0.07 ^{ab}
Old leaf	+B	0.76±0.04 ^{a}	0.72±0.04 ^{a}	0.79±0.06 ^{a}	0.99±0.06 ^{a}	0.09±0.01 ^{b}
	−B	0.75±0.02 ^{a}	0.73±0.02 ^{a}	0.73±0.01 ^{ab}	1.01±0.03 ^{a}	0.19±0.03 ^{a}

Table 2. Activity of nitrate reductase (μmol mg^{-1} pro.), concentration of nitrate (μmol g^{-1} FW) and nitrite (nmol g^{-1} FW), free amino acids (μmol g^{-1} FW) and protein (mg g^{-1} FW) in the young and old leaves and roots of B-sufficient (+B) and B-deficient (−B) tea (*Camellia sinensis* L.). Data in each column followed by the same letter are not significantly different (P<0.05)

	Treatments	NR activity	Nitrate	Nitrite	Amino acids	Protein
Young leaf	+B	203±23 ^{bc}	128±5.5 ^{b}	73±3 ^{b}	5.8±1.2 ^{c}	2.7±0.7 ^{a}
	−B	284±83 ^{b}	101±7.2 ^{c}	121±11 ^{a}	5.6±0.4 ^{c}	2.3±0.3 ^{ab}
Old leaf	+B	221±21 ^{bc}	160±8.5 ^{a}	113±18 ^{a}	7.5±0.6 ^{bc}	2.5±0.3 ^{ab}
	−B	159±39 ^{c}	150±8.8 ^{a}	93±15 ^{ab}	6.4±0.4 ^{c}	1.6±0.2 ^{bc}
Root	+B	453±39 ^{a}	13±2.7 ^{d}	82±9 ^{b}	9.9±0.9 ^{b}	1.1±0.4 ^{c}
	−B	450±81 ^{a}	20±6.7 ^{d}	90±5 ^{ab}	16.2±2.6 ^{a}	1.2±0.3 ^{c}

Discussion

Boron requirement of dicots has been suggested to be higher than that of monocots [29], which was attributed to the different cell wall structure in these two group of plants. To our knowledge, there is no published work on B deficiency in tea plants and tolerance of this species to B deficiency has not been investigated. In this work, visual B deficiency symptoms such as leaf curling were rarely observed. Without application of resin, tea plants in the –B nutrient solution did not show any marked change in growth in comparison to +B plants even after 6 weeks treatment. In contrast, a clear growth difference and leaf symptoms could be easily produced in turnip plants grown in the nutrient solution without resin prepared in our lab [19]. It could be concluded that, tea is highly resistant to B deficiency.

Critical B deficiency content was reported to be in the range of 20-70 μg g^{-1} DW for soil grown dicotyledonous plants [28]. In our experiment, the critical B deficiency content was 72.9 μg g^{-1} DW for the young leaf and 97.7 μg g^{-1} DW for the old leaf, if 90% of the maximal shoot dry weight is used as reference point [28]. Higher amount of critical B deficiency obtained for the old leaf, is the result of greater B concentration in this leaf in B sufficient plants. Leaf B concentration seems to be highly related to the overall rate of transpiratory water loss being higher in mature than the young leaves. Decreasing acropetal concentration gradient in response to increasing developmental maturity of leaves as well as a steep gradient in B concentration of a particular leaf from base to the margins and tips have been demonstrated [9].

Regarding photosynthetic pigments, photochemistry and gas exchange, leaves of different age responded differently to B deficiency. Reduction of Chl a/b ratio and rise of carotenoids concentration under B deficiency conditions were observed only in the old leaves.

Reduction of photosynthetic capacity per leaf weight is expected following reduction of Chl a/b ratio. Although photochemical quenching (*qP*) remained unchanged, non-photochemical quenching (*qN*) increased in parallel to increase of carotenoids in the old leaves of B-deficient plants. Non-photochemical fluorescence quenching is one of the mechanisms that prevent or alleviate damage to the photosynthetic apparatus. In this mechanism, excess radiation energy is dissipated as heat in the light harvesting antenna of PSII [33]. Higher native amounts of leaf carotenoids and flavonoids in the old compared with the young leaf may contribute to the protection of old leaves under conditions of excess reducing power expected to occur in B starved plants due to decreased stomatal conductance (see below). Flavonoids involve in detoxification of H_2O_2 [46] and may function in plants to screen harmful radiation [45]. Carotenoids play a photoprotective role not only by quenching of excess excitation energy of triple Chl and/or $^1O_2$ directly, but also via xanthophyll cycle involves thermal dissipation under visible light [6].

The lack of an effect of B starvation on photochemical parameters including F_v/F_m, F'_v/F'_m, ΦPSII and *qP* indicated that the photosynthesis processes conserved their normal activities and thylakoid constituents have not been damaged seriously in B deprived leaves. Similar to our results, B deficiency resulted in only slight changes in the PSII photochemistry in citrus leaves [20]. It was suggested that, for nutrients without direct involvement in the electron transport or Chl synthesis, a close linkage between nutritional status of leaves and spectral characteristics seems unlikely [1]. However, a significant reduction of Hill reaction, electron transfer activity and O_2 evolution due to B deficiency has been reported in sunflower plants [15]. Such discrepancy could be the result of differences among species in the structural or functional susceptibility of chloroplasts or the extent of production of and/or scavenging reactive oxygen species.

In contrast to photosynthetic pigments and photochemistry, gas exchange parameters in the young leaf responded more prominently to B deficiency as compared to those in the old leaf. The rates of net assimilation and transpiration were diminished in the young leaf of B-deficient plants in parallel with significant reduction of stomatal conductance. Reduction of photosynthesis in the young leaf occurred mostly due to stomatal limitation while in the old leaf mainly through reduction of leaf Chl a/b ratio. In addition, the remarkable reduction of total plant leaf area likely affects whole plant photosynthesis, contributing to the low biomass production under B deficiency conditions. Impaired stomatal conductance under B deficiency conditions was reported for other plant species (18, 16]. Role of B in stomatal opening has not been investigated. Boron is required for membrane integrity [10], function and activity of H^+-ATPase [37, 41] and expression of H^+-ATPase gene [11]. Therefore, it is plausible that B deficiency causes reduction of K^+ uptake into guard cells and following loss of membrane integrity, stimulates passive leakage of K^+ from guard cells. Boron deficiency-induced K^+ leakage from leaf tissues was observed in turnip plants [19].

Boron deficiency increased slightly NR activity in the young leaf accompanied by significantly lower nitrate concentration. In the old leaf, however, NR activity decreased slightly and nitrate concentration remained unchanged. Similar with the old leaf in our experiment, concentration of nitrate and activity of NR was lower in B-deficient tobacco plants compared with control [11]. In contrast to nitrate, nitrite concentration of the young leaf was higher in B-deficient plants likely because of lower nitrite reductase activity. Published studies reporting the influence of stress factors on nitrite reductase activity are rare. Negative influence of salinity

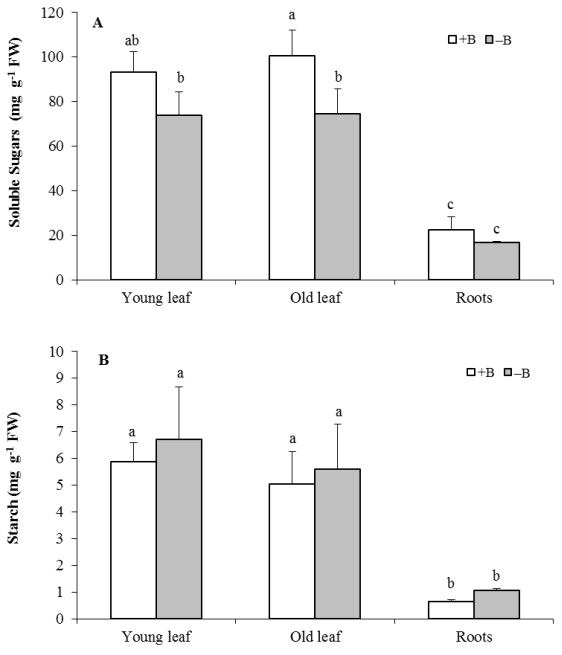

Figure 3. Concentration of soluble sugars (mg eq. glucose g^{-1} FW) (A) and starch (mg g^{-1} FW) (B) in the young and old leaves and roots of B-sufficient (+B) and B-deficient (−B) tea (*Camellia sinensis* L.) plants. Bars indicated by the same letter are not significantly different ($P<0.05$).

on nitrite reductase activity was lower than that on NR activity [36]. In contrast to nitrate and nitrite concentration, free amino acids content was not influenced by supply level in the young leaf. In many cases no correspondence was found between nitrite loss and ammonia production [23]. Accumulation of nitrite in the young leaf is expected to cause metabolic disturbances under B starvation.

Higher activity of NR in combination with significantly lower nitrate concentration in the roots as compared with leaves may suggest that in tea plants nitrate assimilation is mainly occurred in the roots. Striking differences exist between plant species in the proportion of nitrate reduced in the roots. However, a general pattern between plant species in partitioning of nitrate reduction and assimilation between roots and shoots can hardly be suggested. Tropical and subtropical annual and perennial species tend to reduce large proportion of nitrate in shoots with exception observed in open forest and wooding species in Australia and South America [28]. More detailed works are needed for characterization of the partitioning of nitrate reduction in tea plants.

Changes in the activity of antioxidant enzymes due to B deficiency were highly dependent on plant organs and enzyme. Changes observed in the activity of enzymes under B deficiency conditions suggested that, protective effect of APX was likely important for the old leaf and of POD for roots, while SOD contributed to the protection of all three organs against oxidative stress provoked by B deficiency conditions. However, greater activity of antioxidant enzymes did obviously not provide sufficient protection from oxidative damage to membranes as judged by higher accumulation of MDA in both young and old leaves under B deficiency conditions. Nevertheless, H_2O_2 content of leaves and roots did not increase or even decreased under B deficiency conditions implying an efficient H_2O_2 detoxification by antioxidant enzymes or metabolites e.g. by proline [44, see below].

Production of excess amounts of ROS is a common feature of plants under stresses. Oxidative stress has been reported under B deficiency in other plant species [18, 20]. Under deficiency conditions of nutrients involving directly in ROS metabolism e.g. Zn, higher ROS accumulation is plausible. However, under deficiency of nutrients without direct role in antioxidant defense system, indirect effects on ROS metabolism are likely to occur. Accordingly, oxidative damage could be regarded as an indirect effect of B deficiency influencing dry matter production of plants. In this work, oxidative damage in leaves as judged by MDA accumulation under B deficiency, is likely the consequence of imbalance between photosynthetic production of carbohydrates and their use in growth, so the photosystem electron transport chain becomes over-reduced and leads to accelerated production of ROS [20]. Accordingly, in the roots reduction of MDA was observed under B deficiency conditions resulted likely from lower production and/or more efficient scavenging of ROS following elevated activity of POD or SOD.

Proline was predominantly higher in the leaves and roots of B-deficient plants. Proline accumulation often occurs in plants under variety of stress conditions, but a consensus has not emerged on its role in tolerance to stresses. Some researchers assume that proline accumulation is a symptom of injury which does not confer protection against stresses [27]. On the contrary, a relationship between lipid peroxidation and proline accumulation was reported in plants subjected to diverse kinds of stress [32]. Proline acts as a free radical scavenger to protect plants away from damage by oxidative stress [2, 44]. In this work, enhanced accumulation of proline under B deficiency conditions is likely the main cause for lower H_2O_2 content in B deprived plants. Accumulation of proline may be regarded as a strategy of B-deficient tea plants for protection against free radicals and contribute significantly to the higher tolerance of tea plants to B deficiency as compared with susceptible species.

Table 3. Specific activity of ascorbate peroxidase (APX; µmol mg^{-1} protein min^{-1}), peroxidase (POD; µmol mg^{-1} protein min^{-1}), superoxide dismutase (SOD; U mg^{-1} protein) and concentration of malondialdehyde (MDA; nmol g^{-1} FW), hydrogen peroxide (µmol g^{-1} FW) and proline (nmol g^{-1} FW) in the young and old leaves and roots of B-sufficient (+B) and B-deficient (−B) tea (*Camellia sinensis* L.) plants. Data of each enzyme or metabolite followed by the same letter are not significantly different ($P<0.05$)

	Treatments	Young leaf	Old leaf	Root
APX	+B	83±19 ^{b}	88±21 ^{b}	70±4 ^{b}
	−B	44±12 ^{c}	127±9 ^{a}	87±11 ^{b}
POD	+B	8±3 ^{c}	17±4 ^{c}	62±21 ^{b}
	−B	17±3 ^{c}	23±6 ^{c}	175±12 ^{a}
SOD	+B	1.5±0.4 ^{cd}	1.4±0.0 ^{d}	0.8±0.4 ^{d}
	−B	3.2±0.4 ^{b}	5.3±0.8 ^{a}	2.7±0.9 ^{bc}
MDA	+B	29±2 ^{c}	46±5 ^{b}	56±3 ^{a}
	−B	55±1 ^{a}	60±4 ^{a}	34±1 ^{c}
H_2O_2	+B	6.0±1.2 ^{a}	3.8±0.6 ^{b}	1.2±0.2 ^{c}
	−B	3.3±0.5 ^{b}	3.5±0.2 ^{b}	0.6±0.2 ^{c}
Proline	+B	54±7 ^{c}	65±8 ^{bc}	60±8 ^{c}
	−B	87±9 ^{b}	114±9 ^{a}	95±19 ^{ab}

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