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ARTICLE

Effect of light intensity on photosynthesis and antioxidant defense in boron deficient tea plants

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ABSTRACT *Tea* (*Camellia sinensis* (L.) O. Kuntze) plants were grown at adequate (46 μM) or low (<2.5μM) boron (B) supply in the nutrient solution under low (LL, 50 μmol $m^{-2}s^{-1}$), intermediate (IL, 250 μmol $m^{-2}s^{-1}$) and high (HL, 500 μmol $m^{-2}s^{-1}$) light intensities. Boron deficiency hardly affected photochemical events under LL conditions, but alleviated reduction of efficiency of photosynthetic energy conversion in IL and HL plants. The optimum light intensity for CO_2 assimilation was IL for the young and HL for the old leaves. Activity of ascorbate peroxidase and superoxide dismutase and concentration of proline was lower under IL compared to LL and HL conditions. Compared to the old leaves, in the young leaves photochemical events were more protected under excess light and low B supply. Antioxidant defense system involved in the protection of leaves against excess light under IL conditions while thermal dissipation performed this role under HL conditions. Alleviation of high light stress effect on the photochemical events could be attributed to the B deficiency-induced activation of antioxidant defense system.
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KEY WORDS

Camellia sinensis
high light stress
leaf photochemistry
micronutrient deficiency
proline

Boron (B) is an essential micronutrient required for normal growth of higher plants. However, its function in plants has not yet been fully understood (Mengel and Kirkby 2002). Boron is involved in different processes such as vegetative growth, tissue differentiation, metabolic control through regulation of enzymatic reactions, membrane integrity and function, phenolic metabolism, sugar translocation, and nucleic acid synthesis.

It has been shown that B deficiency decreases plant photosynthetic capacity (Zhao and Oosterhuis 2002). Decreased photosynthetic capacity is the result of decreased Hill reaction activity and low intercellular CO_2 concentration (Sharma and Ramchandra 1990), reduced chlorophyll (Chl) content, photosynthetic electron transport rate, photophosphorylation (Plesničar et al. 1997) as well as structural damage (El-Shintinawy 1999). In turnip plants, the photosynthesis apparatus conserved its normal activities and thylakoid constituents were not damaged seriously in B-deprived leaves (Hajiboland and Farhanghi 2010). However, strongly reduced stomatal conductance accompanied by reduced leaf chlorophyll content caused a significant photoinhibition in the leaves of B-deficient turnip plants (Hajiboland and Farhanghi 2010).

Oxidative stress is a central factor in abiotic and biotic stress phenomena that occurs when there is a serious imbalance between the production of reactive oxygen species (ROS) and antioxidant defense capacity (Apel and Hirt 2004). Although B is not a constituent of plants antioxidative defense system, B deficiency like imbalance of other nutrients leads to oxidative stress in plants.

High light intensity is one of the important environmental stresses particularly for shade plants. Various mechanisms operate for protection of the photosynthetic apparatus against damage from the accumulation of excessive energy. Shade plants have low capacities not only for photosynthetic electron transport but also for photoprotective responses such as thermal energy dissipation. In some cases, light stress does not result from high light per se, but rather from an excess of absorbed light beyond that utilized in photosynthesis e.g. in response to factors cause stomatal closure (Demmig-Adams and Adams 1992). Apart from the effects on photosynthesis, phenolics metabolism (Mole and Waterman 1988) and the balance between production and scavenging of ROS (Demmig-Adams and Adams 1992) could be greatly influenced in plants exposed to high light intensity.

Nutritional status of plants has great influence on the tolerance of plants to environmental stresses (Marschner 1995). Because of a wide spectrum of the effect of B deficiency on physiological processes of plants, it is expected that responses of plants to stress factors such as high light intensity would be influenced greatly by their B nutritional status.

Tea (*Camellia sinensis* (L.) O. Kuntze) is cultivated in humid and subhumid of tropical, subtropical and temperate regions of the world. Tea is a shade plant and a canopy of moderate shade provided by shade trees is necessary for an optimum growth and productivity in tea gardens (Janendra

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et al. 2008). Boron deficiency is generally related to high rainfall areas and acid soil conditions common in soils of tea plantations (Shorrocks 1997). To our knowledge, there is hardly any information on the responses of tea plants neither to B deficiency nor to high light stress combined with B starvation.

The objective of this work was study of the effect of low B supply on growth and photosynthesis of tea plants grown under three different lighting conditions. Possible involvement of antioxidant defense system in the response of plants to interaction of low B supply with high light stress was also evaluated.

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 dark and moistened by distilled water and saturated $CaSO_4$ every day. After the emergence of primary leaves, seedlings were transferred to the light. One month-old seedlings were transferred to the nutrient solution (Ghanati et al. 2005) pH 4.2 containing either low (–B) or adequate (46 µM) B supply (+B) and were grown under three photosynthetic photon flux density (PPFD) conditions including low light (LL, 50 µmol $m^{-2}s^{-1}$ PPFD), intermediate light (IL, 250 µmol $m^{-2}s^{-1}$ PPFD) and high light (HL, 500 µmol $m^{-2}s^{-1}$ PPFD) intensities. The incident PPFD was measured by a quantum sensor attached to the leaf chamber of the gas exchange unit. In order to minimize the B contamination of nutrient solution in –B treatment, 1g L^{-1} of washed B specific resin (Amberlite IRA 743, Fluka; Asad et al. 1997) 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 under controlled environmental conditions with a temperature regime of 25°/18°C day/night, 14/10 h light/dark period and a relative humidity of 70/80%.

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 5h, 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 (1982). The second youngest, fully expanded leaf together with the third old leaf defined as young and old leaves respectively, were used for all analyses. 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. An average of 4 records from different parts of each individual leaf was considered for each replicates. Definitions and calculations were described elsewhere (Hajiboland and Amirazad 2010). 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 under the same PPFD as that of the treatments.

Determination of chlorophyll, carotenoids, anthocyanins and flavonoids

Leaf concentration of Chl a, b and carotenoids were determined according to Lichtenthaler and Wellburn (1985) after extraction of pigments in cold acetone and allowing the samples to stand for 24 h in the dark at 4ºC. Anthocyanins and total flavonoid content of leaves were determined according to the methods described before (Hajiboland and Amirazad 2010).

Assay of antioxidant enzymes and related metabolites

Determination of peroxidase (POD, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11) and superoxide dismutase (SOD, EC 1.15.1.1) activity, the amount of malondialdehyde (MDA), H_2O_2 and proline were undertaken according to optimized protocols described elsewhere (Hajiboland and Hasani 2007). Fresh samples were ground in the presence of liquid nitrogen and measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany). Soluble proteins were determined as described by Bradford (1976) using a commercial reagent (Sigma) and BSA (Merck) as standard.

Experiments were 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

Plants dry matter production was increased with increasing light intensity from LL to IL irrespective to the B supply level. Further increase of light intensity from IL to HL did not result in further growth improvement with the exception of root growth of low B plants. Under HL conditions, low B supply did not influence root DW negatively (Fig. 1).

Boron concentration increased with increasing light intensity from LL to IL in leaves but not in roots. In plants grown under HL conditions, B concentration was slightly or significantly lower than IL plants. The same trend was

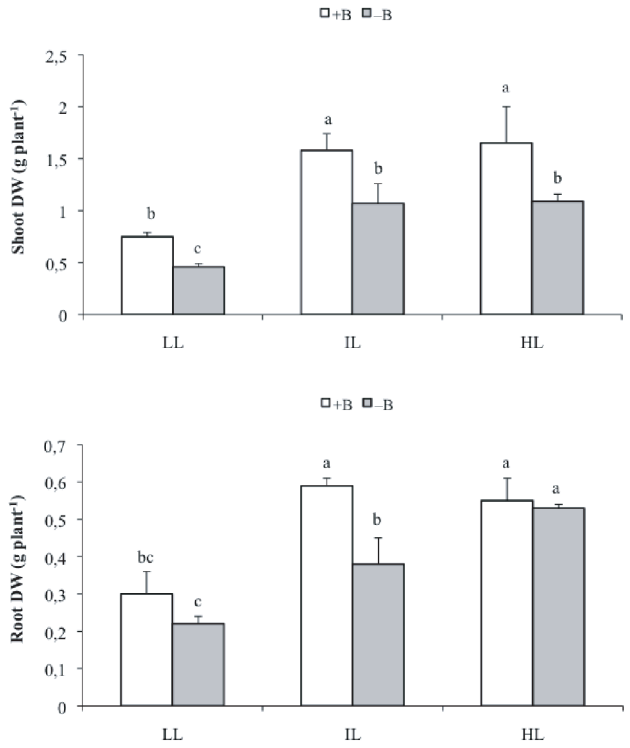

Figure 1. Dry weight (mg plant^{-1}) of shoot and root in tea (*Camellia sinensis* L.) plants grown for six weeks at adequate (+B) or low (–B) boron supply under low (LL, 50 µmol m^{-2}s^{-1}), intermediate (IL, 250 µmol m^{-2}s^{-1}) or high (HL, 500 µmol m^{-2}s^{-1}) light intensity. Bars indicated by the same letter are not significantly different (P<0.05).

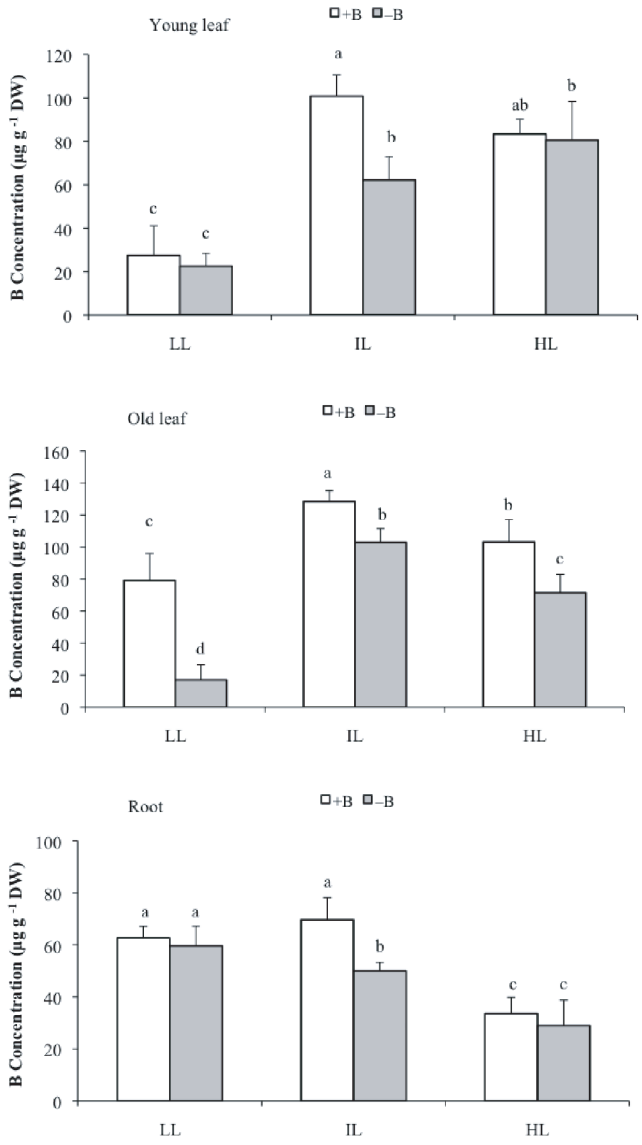

Figure 2. Boron concentration (µg g^{-1} DW) of three different fractions in tea (*Camellia sinensis* L.) plants grown for six weeks at adequate (+B) or low (–B) boron supply under low (LL, 50 µmol m^{-2}s^{-1}), intermediate (IL, 250 µmol m^{-2}s^{-1}) or high (HL, 500 µmol m^{-2}s^{-1}) light intensity. Bars indicated by the same letter are not significantly different (P<0.05).

observed for B content (µg fraction^{-1}) because of parallel changes in the dry weight and B content of fractions under all applied treatments (data not shown).

Under IL and HL compared with LL conditions, concentration of both Chl a and b decreased irrespective to the B nutritional status in both young and old leaves. In LL plants, Chl a and b concentration increased due to low B supply in the young leaf. In the old leaf, however, significant effect of B supply level was observed under IL conditions. In IL and to a lesser extent in HL plants, Chl a content of the old leaf decreased while that of Chl b increased resulting in a reduction of Chla/b ratio from 2.69 to 0.94 in IL and from 3.0 to 2.1 in HL plants due to B deficiency. Leaf carotenoids decreased with increasing light intensity from LL to IL slightly or significant irrespective to the B nutrition of plants. Rising light intensity to HL caused a slight increase of leaf carotenoids in the young and a significant reduction in the old leaf. Boron deficiency caused a significant increase of carotenoids in the young leaf under LL and in the old leaf under IL conditions. Increasing light intensity from LL to IL caused increase in anthocyanins in B-sufficient but not in B-deficient leaves. Under HL conditions, anthocyanins content decreased again. Anthocyanins content of both young and old leaves increased under LL conditions due to low B supply. In general, light intensity and B supply level did not affect flavonoids content of leaves. The exception was significantly lower flavonoids content in the young leaf of B-sufficient HL plants compared with B-deficient ones (Table 1).

In the young leaf, the maximal photochemical efficiency of PSII (F_v/F_m) decreased with increasing light intensity in B-sufficient but not in B-deficient plants. In the old leaf, reduction of F_v/F_m was observed following increased light intensity in both B-sufficient and B-deficient plants. Excita-

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) in young and old leaves of tea (*Camellia sinensis* L.) plants grown for six weeks at adequate (+B) or low (–B) boron supply under low (LL, 50 µmol $m^{-2}s^{-1}$), intermediate (IL, 250 µmol $m^{-2}s^{-1}$) or high (HL, 500 µmol $m^{-2}s^{-1}$) light intensity. Data in each column within each organ followed by the same letter are not significantly different (P<0.05).

Young leaf	Treatments	Chl a	Chl b	Carotenoids	Anthocyanins	Flavonoids
LL	+B	2.1±0.4 ^{b}	0.9±0.0 ^{b}	191±39 ^{b}	7.4±1.7 ^{b}	0.66±0.07 ^{a}
	–B	3.0±0.5 ^{a}	1.4±0.2 ^{a}	267±44 ^{a}	23.1±4.0 ^{a}	0.65±0.09 ^{a}
IL	+B	1.1±0.1 ^{c}	0.4±0.1 ^{d}	86±10 ^{c}	26.0±2.3 ^{a}	0.55±0.07 ^{a}
	–B	1.2±0.3 ^{c}	0.5±0.1 ^{cd}	100±18 ^{c}	21.6±3.2 ^{a}	0.65±0.07 ^{a}
HL	+B	1.8±0.3 ^{bc}	0.7±0.1 ^{bc}	134±24 ^{bc}	4.8±2.3 ^{b}	0.35±0.05 ^{b}
	–B	1.1±0.5 ^{c}	0.6±0.1 ^{c}	130±26 ^{bc}	2.8±1.3 ^{b}	0.52±0.04 ^{a}
Old leaf	Treatments	Chl a	Chl b	Carotenoids	Anthocyanins	Flavonoids
LL	+B	4.5±0.4 ^{a}	1.5±0.2 ^{ab}	320±44 ^{ab}	5.2±1.6 ^{b}	0.89±0.19 ^{a}
	–B	5.1±0.5 ^{a}	1.8±0.1 ^{a}	371±32 ^{a}	27.2±5.1 ^{a}	1.07±0.23 ^{a}
IL	+B	3.5±0.3 ^{b}	1.3±0.1 ^{b}	254±19 ^{b}	29.6±6.2 ^{a}	0.83±0.13 ^{a}
	–B	1.6±0.5 ^{c}	1.7±0.3 ^{a}	331±48 ^{a}	20.9±6.3 ^{a}	0.89±0.10 ^{a}
HL	+B	2.1±0.2 ^{c}	0.7±0.1 ^{c}	149±16 ^{c}	10.9±3.6 ^{b}	0.76±0.04 ^{a}
	–B	1.7±0.3 ^{c}	0.8±0.1 ^{c}	162±8 ^{c}	22.3±2.3 ^{a}	0.78±0.05 ^{a}

Table 2. Chlorophyll fluorescence parameters including F_v/F_m (maximal photochemical efficiency of PSII), F'_v/F'_m (excitation capture efficiency of open PSII), q_P (photochemical quenching), q_N (non-photochemical quenching) and Φ_{PSII} (quantum yield of PSII) in young and old leaves of tea (*Camellia sinensis* L.) plants grown for six weeks at adequate (+B) or low (–B) boron supply under low (LL, 50 µmol $m^{-2}s^{-1}$), intermediate (IL, 250 µmol $m^{-2}s^{-1}$) or high (HL, 500 µmol $m^{-2}s^{-1}$) light intensity. Data in each column within each organ followed by the same letter are not significantly different (P<0.05).

Young leaf	Treatments	F_v/F_m	F'_v/F'_m	q_P	q_N	Φ_{PSII}
LL	+B	0.79±0.01 ^{a}	0.72±0.03 ^{b}	0.97±0.03 ^{a}	0.35±0.02 ^{ab}	0.73±0.01 ^{a}
	–B	0.79±0.01 ^{a}	0.70±0.01 ^{bc}	1.00±0.04 ^{a}	0.45±0.04 ^{a}	0.73±0.01 ^{a}
IL	+B	0.74±0.04 ^{b}	0.73±0.03 ^{b}	0.92±0.03 ^{b}	0.14±0.03 ^{c}	0.70±0.03 ^{a}
	–B	0.79±0.01 ^{a}	0.77±0.02 ^{a}	0.89±0.02 ^{b}	0.13±0.04 ^{c}	0.71±0.01 ^{a}
HL	+B	0.74±0.02 ^{b}	0.67±0.03 ^{c}	0.89±0.04 ^{b}	0.38±0.09 ^{ab}	0.62±0.02 ^{c}
	–B	0.77±0.03 ^{a}	0.72±0.02 ^{b}	0.88±0.01 ^{b}	0.29±0.07 ^{b}	0.66±0.02 ^{b}
Old leaf	Treatments	F_v/F_m	F'_v/F'_m	q_P	q_N	Φ_{PSII}
LL	+B	0.80±0.00 ^{a}	0.80±0.01 ^{a}	0.97±0.04 ^{ab}	0.09±0.01 ^{d}	0.79±0.06 ^{a}
	–B	0.78±0.04 ^{a}	0.78±0.02 ^{ab}	0.96±0.02 ^{ab}	0.08±0.02 ^{d}	0.76±0.01 ^{a}
IL	+B	0.76±0.04 ^{ab}	0.72±0.04 ^{b}	0.98±0.03 ^{ab}	0.09±0.01 ^{d}	0.79±0.06 ^{a}
	–B	0.75±0.02 ^{ab}	0.73±0.02 ^{b}	1.00±0.03 ^{a}	0.19±0.05 ^{c}	0.73±0.01 ^{a}
HL	+B	0.61±0.03 ^{c}	0.39±0.04 ^{c}	0.85±0.02 ^{c}	0.73±0.08 ^{a}	0.34±0.05 ^{c}
	–B	0.72±0.03 ^{b}	0.58±0.02 ^{d}	0.93±0.03 ^{b}	0.59±0.07 ^{b}	0.55±0.02 ^{b}

tion capture efficiency of open PSII (F'_v/F'_m) decreased by higher light intensity slightly or significant in both young and old leaves irrespective to the B nutritional status. Photochemical quenching (q_P) in the young leaf decreased by higher light intensity. Boron supply level did not affect q_P with the exception of the old leaf in HL plants. The lowest amount of non-photochemical quenching (q_N) was detected under IL conditions. Low B supply reduced this parameter only under HL conditions which was slightly in the young and significant in the old leaf. Quantum yield of PSII (Φ_{PSII}) did not respond to either low B supply or light intensity from LL to IL. However, following exposure of plants to HL conditions, reduction of Φ_{PSII} in both young and old leaves was observed. Φ_{PSII} was higher in B-deficient than B-sufficient HL plants. Reduction of F_v/F_m, F'_v/F'_m, q_P and Φ_{PSII} in HL compared to LL plants were 24%, 51%, 12% and 57% in the old leaf respectively. The corresponding values for the young leaf were considerably lower, 6%, 7%, 8% and 15% respectively (Table 2).

In the young leaf, increasing light intensity from LL to IL increased CO_2 net assimilation rate (A), however, A was not further changed significantly with further increase of light to HL intensity. In the old leaf, in contrast, HL plants have significantly higher A than the IL ones. Boron-deficient leaves had significantly or slightly lower A irrespective to the lighting conditions. In the young leaf, a continues reduction of transpiration rate (E) was observed with increasing light intensity and under B deficiency conditions. In contrast,

Table 3. Gas exchange parameters including net photosynthetic rate (*A,* μmol m^{-2} s^{-1}), transpiration rate (*E,* mmol m^{-2} s^{-1}) and stomatal conductance to water vapor (g_s, (mol m^{-2} s^{-1}) in young and old leaves of tea (*Camellia sinensis* L.) plants grown for six weeks at adequate (+B) or low (–B) boron supply under low (LL, 50 μmol $m^{-2}s^{-1}$), intermediate (IL, 250 μmol $m^{-2}s^{-1}$) or high (HL, 500 μmol $m^{-2}s^{-1}$) light intensity. Data in each column within each organ followed by the same letter are not significantly different (P<0.05).

Young leaf	Treatments	*A*	E	g_s
LL	+B	1.88±0.24 ^{bc}	2.96±0.27 ^{a}	0.01±0.00 ^{b}
	–B	1.73±0.11 ^{c}	0.70±0.14 ^{d}	0.01±0.00 ^{b}
IL	+B	3.31±0.27 ^{a}	1.94±0.19 ^{b}	0.54±0.20 ^{a}
	–B	2.36±0.20 ^{b}	0.53±0.24 ^{d}	0.04±0.02 ^{b}
HL	+B	3.77±0.16 ^{a}	1.42±0.31 ^{c}	0.37±0.14 ^{a}
	–B	1.92±0.26 ^{bc}	0.38±0.19 ^{d}	0.06±0.01 ^{b}
Old leaf	Treatments	*A*	E	g_s
LL	+B	1.63±0.21 ^{b}	0.53±0.15 ^{bc}	0.01±0.00 ^{c}
	–B	0.67±0.08 ^{d}	0.30±0.09 ^{c}	0.01±0.00 ^{c}
IL	+B	1.32±0.06 ^{bc}	0.53±0.06 ^{bc}	0.01±0.00 ^{c}
	–B	1.10±0.05 ^{c}	0.36±0.11 ^{bc}	0.02±0.00 ^{c}
HL	+B	2.48±0.28 ^{a}	1.39±0.11 ^{a}	0.28±0.02 ^{b}
	–B	1.48±0.09 ^{b}	0.57±0.13 ^{b}	0.32±0.02 ^{a}

Table 4. Specific activity of peroxidase (POD, μmol mg^{-1} protein min^{-1}), ascorbate peroxidase (APX, μmol mg^{-1} protein min^{-1}) and superoxide dismutase (SOD, U mg^{-1} protein) in young and old leaves and roots of tea (*Camellia sinensis* L.) plants grown for six weeks at adequate (+B) or low (–B) boron supply under low (LL, 50 μmol $m^{-2}s^{-1}$), intermediate (IL, 250 μmol $m^{-2}s^{-1}$) or high (HL, 500 μmol $m^{-2}s^{-1}$) light intensity. Data in each column within each organ followed by the same letter are not significantly different (P<0.05).

Young leaf	Treatments	POD	APX	SOD
LL	+B	32±9 ^{a}	124±7 ^{bc}	3.4±0.6 ^{b}
	–B	35±3 ^{a}	171±37 ^{a}	6.4±0.4 ^{a}
IL	+B	8±2 ^{c}	83±19 ^{cd}	1.5±0.4 ^{c}
	–B	17±3 ^{b}	44±12 ^{d}	3.2±0.4 ^{b}
HL	+B	18±6 ^{bc}	105±2 ^{c}	2.8±0.3 ^{b}
	–B	5±3 ^{d}	164±9 ^{ab}	3.4±0.7 ^{b}
Old leaf	Treatments	POD	APX	SOD
LL	+B	18±3 ^{a}	128±19 ^{b}	6.5±0.2 ^{a}
	–B	23±2 ^{a}	169±10 ^{a}	1.3±0.7 ^{e}
IL	+B	17±9 ^{a}	88±21 ^{cd}	1.4±0.0 ^{de}
	–B	23±9 ^{a}	127±9 ^{b}	5.3±0.8 ^{b}
HL	+B	19±2 ^{a}	116±20 ^{bc}	3.4±0.3 ^{c}
	–B	19±4 ^{a}	78±16 ^{d}	2.5±0.6 ^{cd}
Root	Treatments	POD	APX	SOD
LL	+B	186±34 ^{ab}	123±11 ^{cd}	5.0±1.2 ^{b}
	–B	214±18 ^{a}	197±15 ^{b}	9.0±1.9 ^{a}
IL	+B	62±21 ^{d}	70±4 ^{d}	0.8±0.4 ^{d}
	–B	175±12 ^{ab}	87±11 ^{d}	2.7±0.4 ^{bc}
HL	+B	151±44 ^{bc}	299±82 ^{a}	3.7±0.7 ^{bc}
	–B	109±23 ^{cd}	250±28 ^{ab}	1.4±0.3 ^{cd}

higher light intensity (500 μmol $m^{-2}s^{-1}$) resulted in greater E in the old leaf and B deficiency effect was significant only in HL plants. Somatal conductance (g_s) in the young leaf increased with increasing light intensity from LL to IL, but was not influenced further at HL conditions. In the old leaf, however, a continuous increase in g_s was observed with increasing light intensity up to HL intensity. Boron deficiency resulted in stomatal closure that was significant in the young leaf under IL and HL conditions and in the old leaf only under HL conditions (Table 3).

Activity of all three studied antioxidant enzymes decreased with increasing light intensity from LL to IL slightly or significant irrespective to the B supply level. Only activity of POD in the old leaf was not affected either by light conditions or B nutritional status. With further rise of light intensity (at HL) a slight or significant increase in the activity of en-

Table 5. Concentration of malondialdehyde (MDA, nmol g^{-1} FW), hydrogen peroxide (H_2O_2, nmol g^{-1} FW), proline (nmol g^{-1} FW) and protein (mg g^{-1} FW) in young and old leaves and roots of tea (*Camellia sinensis* L.) plants grown for six weeks under adequate (+B) or low (–B) boron supply under low (LL, 100 µmol $m^{-2}s^{-1}$), intermediate (IL, µmol $m^{-2}s^{-1}$) or high (HL, µmol $m^{-2}s^{-1}$) light intensity. Data in each column within each organ followed by the same letter are not significantly different (P<0.05).

Young leaf	Treatments	MDA	H_2O_2	Proline	Protein
LL	+B	43±5 cd	3.4±0.4 b	213±73 a	1.4±0.1 ab
	–B	46±6 bc	4.6±0.4 ab	73±18 b	1.4±0.1 ab
IL	+B	29±2 d	6.0±1.2 a	54±7 b	1.6±0.2 a
	–B	55±1 b	3.3±0.5 b	87±9 b	1.6±0.2 a
HL	+B	56±9 b	5.1±1.4 ab	78±17 b	1.2±0.1 ab
	–B	76±7 a	5.0±0.4 ab	183±18 a	1.0±0.3 b
Old leaf	Treatments	MDA	H_2O_2	Proline	Protein
LL	+B	19±4 c	6.8±0.4 a	374±59 a	1.4±0.2 a
	–B	48±4 b	5.5±0.7 b	206±47 b	1.2±0.1 a
IL	+B	46±5 b	3.8±0.6 cd	65±8 d	1.4±0.2 a
	–B	60±4 b	3.5±0.2 cd	114±9 cd	0.7±0.1 b
HL	+B	99±5 a	4.6±0.9 bc	108±36 cd	1.4±0.1 a
	–B	111±18 a	2.6±0.2 d	166±18 bc	1.5±0.2 a
Root	Treatments	MDA	H_2O_2	Proline	Protein
LL	+B	19±5 d	0.4±0.1 c	151±32 a	1.0±0.1 a
	–B	322±6 a	0.5±0.1 c	88±14 bc	0.7±0.2 ab
IL	+B	56±3 b	1.2±0.2 b	60±8 bc	0.8±0.0 a
	–B	34±1 c	0.6±0.2 c	95±19 b	0.7±0.2 ab
HL	+B	40±3 c	1.6±0.0 a	51±6 c	0.4±0.1 b
	–B	35±7 c	1.1±0.1 b	72±4 bc	0.8±0.2 a

zymes was observed. Boron deficiency, in general, increased activity of antioxidant enzymes with the exception of POD activity in the young leaf of HL plants (Table 4).

Malondialdehyde (MDA) content in the young leaf decreased with increasing light intensity from LL to IL and increased again with exposure of plants to HL conditions. In the old leaf, however, a continues increase was observed with increasing light intensity. Boron deficiency caused mainly significant or slight increase of MDA content. Concentration of H_2O_2 increased continuously with increasing light intensity in the young leaf and roots, while decreased in the old leaf. Boron deficiency either caused no significant effect on H_2O_2 content or reduced it in all three examined fractions. Exposure of plants to higher light intensity declined proline content of leaves and roots. Boron deficiency caused a significant reduction of proline content in LL plants. In IL and HL plants, in contrast, B deficiency resulted in significant or slight increase of proline in all three tested fractions (Table 5).

Discussion

Considering growth responses of plants to increasing light intensity, it could be suggested that 250 µmol m^{-2} s^{-1} is an optimum light intensity for growth of tea plants. Light intensity of 500 µmol m^{-2} s^{-1} did not lead to greater dry matter production even though was not high enough to act as a stress factor if judged on the basis of dry weight data.

Boron concentration of plants increased with increasing light intensity from LL to IL conditions that could be attributed to increase in transpiration following rise of stomatal conductance. Leaf B concentration seems to be highly related to the overall rate of transpiratory water loss (Marschner 1995). However, slight or significant reduction of leaf B concentration with further increase of light intensity e.g. in HL plants, could be explained by parallel reduction of stomatal conductance only in the young leaf. The cause for reduction of B concentration in the old leaf under HL conditions though increase of stomatal opening is not known and may be the result of re-translocation of B to the young leaves. We observed that mature leaves of tea plants are capable to re-translocate B into young leaves particularly under B deficiency conditions (Hajiboland, et al. unpublished data).

A continues reduction of Chl content with increasing light intensity being more pronounced in the old compared with the young leaf, could be attributed to Chl destruction and degeneration of plastid membranes. On the other hand, under HL conditions concentration of anthocyanins in the young leaf was considerably lower than IL or LL conditions irrespective to the B nutritional status. Young leaves of tea plants similar with many other species are transiently red because of the accumulation of anthocyanins. Two potential functions have been proposed for the foliar anthocyanins including sunscreen photoprotective function against excess visible light and making them less discernible to insect herbivores (Karageorgou and Manetas 2006). The anthocyanin accumulation is thought to mask Chl and/or act as a filter for preventing high light absorption by leaves and thus minimize

photoinhibition (Farrant 2000). Destruction of Chl and anthocyanins under increasing light intensity is obviously one of important reasons for and/or results of susceptibility of tea plants to higher light intensity.

Boron deficiency did not reduce Chl content of the young leaf, conversely, a significant increase was observed under LL conditions. It could be attributed to reduced growth and expansion of the young leaf, therefore, concentration effect. Similarly, increased carotenoids and anthocyanins concentration of both young and old leaves observed particularly in IL plants was likely a concentration effect due to growth impairment of low B leaves. However, for anthocyanins higher synthesis is also an explanation because of the well known accumulation of phenolics in B-deficient plants (Marschner 1995). In contrast, reduction of Chl a and Chl a/b ratio of the old leaf due to B deficiency observed particularly under IL conditions was likely the result of decreased synthesis and/or damage to the plastid membranes.

Significant reduction of maximal photochemical efficiency of PSII (F_v/F_m), excitation capture efficiency of open PSII (F'_v/F'_m), photochemical quenching (q_P) and quantum yield of PSII (Φ_{PSII}) with increasing light intensity indicated the occurrence of photoinhibition and/or a serious damage to PSII. It was suggested that, prior to the occurrence of any damaging processes, photoinhibition takes place which can result from an increase in thermal energy dissipation as a photoprotective process and associated with increase in the pool size of xanthophyll cycle pigments (Demmig-Adams and Adams 1992). Non-photochemical fluorescence quenching (q_N) is a mechanism to prevent or alleviate damage to the photosynthetic apparatus (Müller et al. 2001). In IL plants of our experiment, reduction in the efficiency of photosynthetic energy conversion compared to LL plants was associated with reduction of leaf carotenoids and q_N particularly in the young leaves leading likely to PSII damage. With increasing light intensity, leaf carotenoids content and q_N values increased again caused likely protection of leaves from serious damages. In this case, decrease in PSII efficiency resulted mainly from photoprotective increase in thermal energy dissipation induced by excess of adsorbed light (Demmig-Adams and Adams 1992). It implied that, photosynthetic apparatus in both young and old leaves are more protected against excess energy under high compared with intermediate light intensities.

With a lowered capacity for photosynthetic CO_2 assimilation in B-deficient leaves as the consequence of stomatal closure, the requirement for reducing power and photophosphorylation will be lowered. Accordingly, it was expected the actual PSII efficiency to be down-regulated and surplus excitation energy to be dissipated. However, B-deficient plants had lower qN compared to B-sufficient plants apparently because of improvement in electron transport events without need to thermal energy dissipation. Accordingly, B deficiency alleviated inhibitory effect of excess light on the efficiency of photosynthetic energy conversion in both young and old leaves. One possible mechanism is likely an increased activity of antioxidant enzymes and accumulation of antioxidants such as proline (see below). There are two ways for photoprotection of leaves against excess light, namely removal of excess excitation energy directly within the light-capturing system *i.e.* thermal dissipation, and removal of active oxygen formed in the photochemical apparatus by various components of antioxidant defense system (Demmig-Adams and Adams 1992). It seems likely that, protection of photosynthetic apparatus in tea plants under intermediate light intensity is mainly achieved by antioxidant defense system while thermal dissipation mechanisms act profoundly under higher light intensity.

Our results demonstrated that, the inhibitory effect of increasing light intensity on the leaf photosynthetic pigments and photochemical events was much more expressed in the old leaves. Old leaves were also more susceptible against B deficiency-induced damages to photochemistry and Chl a/b ratio compared to the young leaves.

Increasing light intensity led to increased stomatal conductance, transpiration and CO_2 assimilation rate. For the young leaves, IL conditions seemed to be optimum for stomatal conductance, while in the old leaves HL conditions resulted in more opened stomata compared with IL conditions and greater transpiration and assimilation rate. Net assimilation rate per leaf surface area was depressed by low B supply mainly due to stomatal limitation. Impaired stomatal conductance under B deficiency conditions was reported for other plant species (Han et al. 2008; Hajiboland and Farhanghi 2010). Role of B in stomatal opening has not been investigated. Boron is required for membrane integrity (Cakmak and Römheld 1997), function, activity and expression of H^+-ATPase gene (Camacho-Cristóbal and González-Fontes 2007). 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.

In general, activity of antioxidant enzymes was greater in LL than IL conditions. It was likely the cause for reduction of MDA and H_2O_2 in IL compared with LL plants and resulted in the protection of leaf photochemistry though lower efficiency of xanthophylls cycle-related thermal dissipation. With increasing light from 250 to 500 μmol m^{-2} s^{-1}, activity of enzymes increased again, obviously because of production of more active oxygen radicals. Nevertheless, this activation did not lead to reduction of antioxidants and could not provide enough protection against free radicals and cellular damage was occurred apparently. This could be explained well with reduction of photochemical parameters and damage to reaction centers as judged particularly by reduction of F_v/F_m.

Boron deficiency caused mainly activation of antioxi-

dant enzymes that in some cases were effective in reduction of lipid peroxidation and H_2O_2 content. Proline was one of important components in response of tea plants to both light intensity and B deficiency. It was decreased under IL conditions compared with LL, and increased again with increasing light intensity. Indeed, it reflected the optimum light conditions for plants and was correlated well with plants growth response to light intensity. Boron deficiency increased proline concentration only under IL and HL conditions. 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 (Lutts et al. 1996). On the contrary, a relationship between lipid peroxidation and proline accumulation was reported in plants subjected to diverse kinds of stress (Molinari et al. 2007). Proline acts as a free radical scavenger to protect plants away from damage by oxidative stress (Alia and Matysik 2001; Wang et al. 2009). However, excess light responsiveness of proline biosynthesis had not been studied so far. Proline accumulation in plants during stresses such as salinity is controlled by coordinate induction of biosynthesis and inhibition of degradation pathways (Ábrahám et al. 2003). Light dependence of proline synthesis was reported by some authors (Hanson and Tully 1979; Ábrahám et al. 2003). However, effect of high light stress as a factor for increasing proline synthesis has not been studied. Excess light likely exert its effect mainly via blue light absorbing pigments (Briggs and Christie 2002). Effect of excess light on the proline biosynthesis and its role in protection of chloroplast should be investigated further.

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