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Structural, physiological, and biochemical profiling of tea plants under zinc stress

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Abstract

Zinc is the most widespread deficient micronutrient in the tea growing soils of India which affects growth of the plants. In order to investigate the structural, physiological, and biochemical changes under Zn stress (*i.e*. both deficient and excess supply) of tea [*Camellia sinensis* (L.) O. Kuntze cv. T-78] plants, we treated young plants with $ZnSO_4$ at 0 (deficiency), 0.3, 3 (optimum), and 30 μM (toxic) concentrations for 8 weeks. Zn deficiency and excess resulted in considerable decrease in shoot and root fresh and dry masses, and transmission electron microscopy (TEM) revealed disorganization of some cellular organelles. Further, Zn-stress decreased net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and content of chlorophylls *a* and *b*. On the other hand, content of superoxide anion, malondialdehyde, hydrogen peroxide, and phenols, and electrolyte leakage were elevated in stressed plants. The activities of ascorbate peroxidase, catalase, superoxide dismutase, and peroxidase as well as expression of respective genes were up-regulated under Zn-stress. Nevertheless, antioxidant system as a whole did not afford sufficient protection against oxidative damage.

Additional key words: antioxidative enzymes, *Camellia sinensis,* net photosynthetic rate, reactive oxygen species, RT PCR, transpiration rate.

Introduction

Zn is important micronutrient and Zn-deficient leaves become chlorotic or even necrotic under high irradiance (Cakmak 2000). Loss of membrane integrity due to the generation of ROS is a preliminary consequence of Zn deficiency (Cakmak and Marschner 1988). Plasma membrane vesicles of Zn-deficient roots showed higher rates of NAD(P)H oxidation compared to control roots (Pinton *et al*. 1994). Being an integral constituent of Cu/Zn-superoxide dismutase (Cu/Zn SOD), Zn plays a role in detoxification of $O_2^{\bullet -}$ to H_2O_2. Reduced activity of several enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPC/O), aldolase, sucrose synthase, and starch synthetase in plant cells due to Zn starvation have been reported (Marschner 1995). Zn-deficiency increased catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), and superoxide dismutase (SOD) activities in mulberry (Tewari *et al*. 2008). Alternatively, under excess supply of Zn, the chromatin materials condensed, nuclear membrane of cortical cells disrupted, cytoplasm as well as cell organelles disintegrated, vacuoles developed, and number of nucleolus increased (Rout and Das 2003). It was postulated that Zn is also involved in stomatal opening, possibly as a factor in maintaining membrane integrity and K^+ uptake, as well as in CO_2 transport as a constituent of the carbonic anhydrase (Sharma *et al*. 1995). However, supra-optimal supply of Zn suppressed the uptake of Mg and Fe in tea (Venkatesan *et al*. 2006). Zn^{2+} was found to displace magnesium (Mg^{2+}) in RuBPC/O which resulted in loss of its activity (Tewari *et al*. 2008).

It has been reported that 48 % of Indian soils are Zn deficient (Singh 2009) and application of phosphate

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Abbreviations: APX - ascorbate peroxidase; Car - carotenoids; CAT - catalase; Chl - chlorophyll; E - transpiration rate; g_s - stomatal conductance; MDA - malondialdhyde; P_N - net photosynthetic rate, POD - peroxidase; SOD - superoxide dismutase.
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reduces Zn availability to plants (Chandra Mouli *et al*. 2012). On the other hand, Zn application increased content of polyphenols, catechins, amino acids, soluble sugars, RNA, nitrate reductase (NR) activity, as well as protein synthesis (Huiqun and Yuchen 1987, Wu and Fang 1994), and reduced malondialdehyde (MDA) content in tea leaves (Yan *et al*. 1997).

Tea is an important and popular beverage and structural, physiological, and biochemical changes under various abiotic stresses have been recently reported (Das and Mondal 2010, Das *et al*. 2012, Mukhopadyay *et al*. 2012) yet it has not been done for Zn stress. Thus, we have undertaken a detailed study to understand the effect of Zn deficiency and excess on structural, physiological, and biochemical changes of young tea plants.

Materials and methods

Two year-old uniform and well-rooted tea [*Camellia sinensis* (L.) O. Kuntze cv. T-78] plants of 45 cm height (raised in sand bed nursery) were transplanted in earthen pots filled with sand moistened with Murashige and Skoog (1962; MS) solution (pH 5.6) of 1/3 strength every alternative day. Seven weeks after transplanting, the Zn stress treatment was applied for 8 weeks until pronounced visual indications of stress (*e.g*. chlorosis, growth impairment, and die-back of shoots emerged). $ZnSO_4$ was added to 1/3 strength MS solution at following concentrations: 0 μM (Zn-deficiency), 0.3, 3.0 μM (Zn-optimum) and 30 μM (Zn-excess) and solutions were applied twice a week. The plants were grown under a 14-h photoperiod, irradiance of 300 μmol m^{-2} s^{-1}, temperature of 25 ± 2 ºC, and relative humidity of 70 %. For each treatment, five pots (with 5 plants) were arranged in a completely randomized block design.

At the end of the experiment, 12 plants per treatment from different pots were harvested. The plants were cut into shoots and roots and weighed. The plant material was then dried at 80 °C for 48 h for measuring dry mass. There were twelve replicates per treatment.

Leaf and root samples were fixed in 2.5 % (m/v) glutaraldehyde solution in 50 mM potassium phosphate (pH 6.8). The samples were kept overnight at room temperature and washed 3 times for 15 min each with 50 mM sodium cacodylate buffer (pH 6.9). After that, samples were diluted (1:1) with 50 mM sodium cacodylate buffer (pH 6.9) for TEM study (Sandalio *et al*. 2001).

Photosynthetic rate (P_N), transpiration (E), and stomatal conductance (g_s) were recorded in 3rd leaf by a portable photosynthesis system (*LCpro+*, *ADC*, Hoddesdon, UK) at ambient CO_2 concentration, irradiance of 1 300 ± 35 μmol m^{-2} s^{-1} between 10:30 and 12:00 on a clear day. During measurements, leaf temperature and ambient vapor pressure deficit were 26.0 ± 1.0 °C and 1.9 ± 0.1 kPa, respectively. There were five replicates per treatment. An average of 4 records from different parts of individual leaf was considered for each replicate.

Amounts of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) were assayed according to Lichtenthaler (1987) in acetone extracts and the absorbance was read at 470, 663, and 645 nm (spectrophotometer *Lambda 25*, *Perkin-Elmer*, Massachusetts, USA). There were five replicates per treatment.

To determine the content of Zn, samples were oven dried (70 ºC for 3 d), ground to a fine powder, and digested in an acid mixture (HNO_3:H_2SO_4:$HClO_4$; 5:1:1) (Allen *et al*. 1986). Digested samples were diluted with deionized double-distilled water and Zn concentrations were determined using an atomic absorption spectrometer (model No. *2380*, *Perkin-Elmer*). Determination of $O_2^{\bullet-}$ was done following the protocol of Jordan and DeVay (1990). Lipid peroxidation was determined by measuring the amount of MDA (Okhawa *et al*. 1979) and generation of H_2O_2 by the protocol of Sagisaka (1976). Membrane permeability was determined following the protocol of Nanjo *et al*. (1999). Determination of soluble sugar content was carried out by anthrone method and absorbance was recorded at 630 nm (Hedge and Hofreiter 1962). Reducing sugar was estimated following the dinitrosalicylic acid method and the absorbance was measured at 510 nm (Miller 1972). For determination of starch, we followed the method of Hedge and Hofreiter (1962). Phenol in tissue was determined on the basis of the reaction with phospho-molybdate in Folin-Ciocalteau reagent under alkaline conditions. It resulted in the formation of a blue colored complex, absorbance of which was measured at 650 nm (Bray and Thorpe 1954).

For determination of enzyme activities, 100 mg of leaf or root tissues were ground with liquid nitrogen and then resuspended in 1 cm^3 of 50 mM Tris-HCl (pH 7.8) buffer fortified with 1 % (m/v) polyvinylpyrrolidone (PVP). Homogenates were centrifuged at 17 000 *g* and 4 ºC for 15 min. The supernatant was used to measure the activities of ascorbate peroxidase (APX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.6), superoxide dismutase (SOD; EC 1.15.1.1), and peroxidase (POD; EC 1.11.1.7). For APX, the solution contained 50 mM phosphate buffer (pH 7.0), 0.5 mM EDTA (*Sigma*, Bangalore, India), 1 mM ascorbic acid (AsA; *Hi-Media*, Bombay, India), and 1 % PVP (*Hi-Media*). APX activity was determined by monitoring the decrease in absorbance at 290 nm (Nakano and Asada 1981). CAT and SOD activities were determined by measuring the absorbance at 240 nm and 560 nm, respectively, according to Upadhyaya and Panda (2004). POD activity was determined following the protocol of Chance and Maehly (1955). There were five replicates per treatment.

Total RNA was extracted from tissues using *TriZol* reagent (*Invitrogen*, Carlsbad, CA, USA) following the manufacturer's instructions. The yield and quality of total

RNA was determined by *Biophotometer plus* (*Eppendorf*, Chennai, India) and agarose gel electrophoresis, respectively. The cDNA was synthesized using 1 μg of RNA with 200 U of reverse transcriptase *Superscript*^{TM} *III* (*Invitrogen*), 10 mM dNTPs and 250 ng of oligo(dT). After standardizing the optimal amplification at exponential phase, PCR was carried out. The 26S rRNA based gene primers (GenBank ID: AY283368) were used as internal control for expression studies. Different gene specific primers (Table 1) were designed based on the respective expressed sequence tags (ESTs) available at *NCBI*. Linearity between the amount of input RNA and the final RT-PCR product were verified and confirmed. The amplified fragments were cloned in *pGEM-T*, sequenced, and their identity to the corresponding EST confirmed.

Each experiment was repeated 3 times with 5 (biomass studies 12) replicates. All quantitative data were subjected to one way analysis of variance (*ANOVA*) for each parameter. The mean differences were evaluated for least significance differences (LSD) ($P < 0.05$) using *Indostat* statistical package (Hyderabad, India).

Table 1. Gene specific primers used for RT-PCR (F - forward, R - reversed).

Gene	Primer	Sequence (5'-3')
26S rRNA	F	ATGAGTAGGAGGGCGCGCGGCGGT
	R	GGAGGCACTCGGTCCTCCGGAT
14-3-3	F	CGTCTACATGGCCAAACTGG
	R	GCTTCTCCTCTTCGTGCTTG
apx	F	AAGAAGGCTATTGACAAAGCCAAG
	R	GCTTCATGTGGGCTTCTGCATA
sod	F	ATGGCTCTTCGGACTCTGTTGAC
	R	GGGCATACCTTTTCATACACTTCAC
cat	F	AGGGGAACTTTGATCTGGTGG
	R	CTCGTCGGTGCATGAAATTCA

Results

Plants grown in the absence of Zn initially developed Zn-deficient symptoms at the shoot apex and in the actively growing leaves. These symptoms included stunted growth, formation of leaves with narrow blades, and inward curling of leaf margins. Apex of some deficient plants displayed formation of rosette leaves. Mature leaves were chlorotic and the plants started flowering and producing seeds. Root branching was found to be marginally sluggish. No such characteristics were observed in plants supplied with 0.3 or 3 μM Zn (data not shown). Alternatively, retarded growth, die-back of shoot tips, defoliation, and root degeneration was observed in the plants supplied with 30 μM Zn. Both deficiency and excess supply decreased shoot and root biomass and Zn-excess plants were more affected (Table 2).

TEM analysis demonstrated electron dense material in vacuoles of Zn-deficient plants (Fig. 1*A*), but plants supplied with 0.3 or 3 μM Zn possessed no deformity in chloroplast or vacuole ultrastructure (Fig. 1*B*,*C*). In contrast, aberrations in the cellular inclusions, chloroplasts with disorganized thylakoid system (Fig. 1*D*), and swelling mitochondria (Fig. 1*E*) were noted in Zn-excess plants.

All parameters related to exchange of gases revealed a steady decline in both Zn deficient and excess plants. Plants treated with 0.3 μM Zn showed 66 % increment in P_N, 73 % in g_s, and 64 % in E compared to the deficient plants. Plants supplied with 3 μM Zn demonstrated 120 % and 79 % increase in g_s, and E, respectively (Table 2).

Whereas amount of Chl *a*, Chl *b*, and their ratio (Chl *a*/*b*) decreased either in Zn deficiency or in excess, content of Car increased in Zn deficiency compared to the rest treatments (Table 2).

Zn content was in the customary range for plants supplied with 0.3 or 3 μM Zn whereas Zn deficiency was reflected by Zn content of the plants barred from Zn supply and excess plants had the maximum quantity of Zn in both the leaf and root (Table 2). $O_2^{\bullet -}$ accumulation was 75 and 79 % higher in leaves and roots of Zn-deficient compared to 3 μM plants Similarly, MDA content increased under Zn-deficiency and Zn-excess (Table 2). However, electrolyte leakage was higher in the

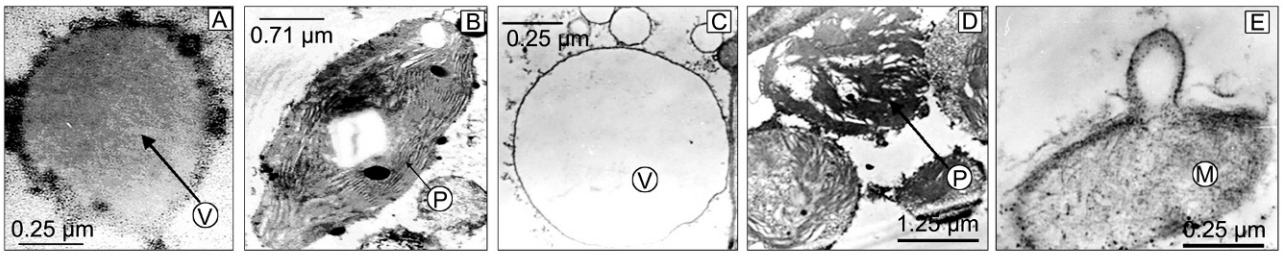

Fig. 1. Ultrastructural changes of the tea leaf and the root under Zn-deficiency and Zn-excess: *A* - vacuole (V) of a palisade parenchyma cell filled with electron dense phenolic compounds at 0 μM Zn; *B* - normal chloroplast (P) at 0.3 μM Zn; *C* - normal vacuole (V) at 3μM Zn; *D* - chloroplast (P) with distorted grana at 30 μM Zn; *E* - mitochondrium (M) with localized swelling at 30 μM Zn.

Table 2. Effects of Zn treatmenst on different characteristics of tea plants (EL - electrolyte leakage, total s. - total sugars, red. s. - reducing sugars, pg - purpurogallin). Means ± SE, $n = 5$. Different letters indicate significant differences at $P \leq 0.05$.

Zn [μM]	0		0.3		3.0		30.0	
Tissue type	leaf	root	leaf	root	leaf	root	leaf	root
FM [g plant^{-1}]	12.75±0.74b	7.19±0.07c	13.73±0.67a	8.37±0.19b	14.70±0.70a	9.32±0.12a	9.02±0.69c	4.04±0.13d
DM [g plant^{-1}]	3.84±0.23a	2.43±0.02ab	4.21±0.15a	2.51±0.06a	4.41±0.13a	2.59±0.02a	3.26±0.10b	1.81±0.02b
P_N [μmol m^{-2} s^{-1}]	8.78±0.03c	-	14.65±0.06a	-	12.38±0.02b	-	7.45±0.01d	-
g_s [mmol m^{-2} s^{-1}]	30.01±0.02c	-	52.01±0.07b	-	66.00±0.02a	-	31.04±0.06c	-
E [mmol m^{-2} s^{-1}]	1.37±0.01c	-	2.25±0.02b	-	2.45±0.02a	-	1.12±0.03d	-
Chl a+*b* [μg g^{-1}(FM)]	1786.6±94.1c	-	2494.8±57.8a	-	2320.5±51.1b	-	1449.7±45.7d	-
Chl *a* [μg g^{-1}(FM)]	1260.8±38.5c	-	1872.2±17.4a	-	1729.7±27.2b	-	945.3±14.8d	-
Chl *b* [μg g^{-1}(FM)]	525.8±16.5c	-	622.5±8.4a	-	590.8±8.8b	-	504.4±12.7b	-
Chl *a*/*b*	2.40±0.01c	-	3.01±0.03a	-	2.93±0.03b	-	1.87±0.03d	-
Car [μg g^{-1}(FM)]	635.9±6.48a	-	527.0±6.01b	-	504.6±5.26c	-	227.8±6.80d	-
Car/Chl	0.36±0.01a	-	0.21±0.02b	-	0.22±0.03b	-	0.16±0.01c	-
Zn [mg kg^{-1}(DM)]	15.30±0.12d	13.08±0.03c	19.10±0.04c	16.00±0.05bc	25.80±0.06b	18.30±0.05b	29.30±0.08a	36.15±0.03a
$O_2^{\bullet-}$ [μmol g^{-1}(FM)]	49.00±0.16a	59.00±1.22a	32.00±0.12c	35.10±0.12d	29.00±0.05d	37.00±0.06c	45.00±0.26b	51.1±0.16b
H_2O_2 [μmol g^{-1}(FM)]	35.00±0.14b	26.00±0.09b	27.00±0.39c	17.10±0.06d	28.00±0.26c	19.10±0.09c	40.10±0.16a	30.06±0.03a
MDA [μmol g^{-1}(FM)]	2.47±0.07b	1.99±0.03b	1.12±0.04c	0.99±0.03d	1.26±0.04c	1.13±0.03c	2.55±0.07a	2.13±0.04a
EL [%]	18.00±0.16b	16.10±0.71b	15.00±0.14d	9.00±0.06c	16.00±0.09c	9.51±0.13c	20.00±1.22a	17.10±0.14a
Total s. [mg g^{-1}(FM)]	7.50±0.06c	3.71±0.04b	11.91±0.06a	6.53±0.07a	10.12±0.03b	6.41±0.07a	5.15±0.05d	3.81±0.06b
Red. s. [mg g^{-1}(FM)]	3.91±0.04c	2.11±0.04b	7.87±0.08a	4.33±0.05a	7.09±0.05b	4.12±0.04a	2.31±0.06d	1.98±0.03b
Starch [mg g^{-1}(FM)]	7.72±0.09c	3.82±0.06b	11.11±0.09a	5.51±0.06a	9.87±0.12b	5.31±0.09a	4.11±0.04d	2.02±0.09c
Phenol [(mg g^{-1}(FM)]	3.80±0.06a	5.51±0.05a	1.90±0.01b	2.12±0.04b	2.33±0.09b	2.21±0.04b	4.34±0.05a	5.60±0.17a
APX [μmol(H_2O_2) g^{-1}(FM) min^{-1}]	3.11±0.01a	2.71±0.01a	1.60±0.02c	1.51±0.02b	1.71±0.01c	1.85±0.01b	2.60±0.03b	0.52±0.04c
CAT [μmol(H_2O_2) g^{-1}(FM) min^{-1}]	703.3±10.0a	358.3±6.4a	582.7±14.2c	197.7±2.2b	471.8±8.0d	181.3±3.4b	891.3±8.4a	202.8±1.4b
SOD [Unit g^{-1}(FM)]	4.20±0.02a	3.80±0.01a	1.60±0.03c	1.40±0.03c	1.63±0.03c	1.44±0.01c	2.48±0.01b	2.51±0.03b
POD [μmol(pg) g^{-1}(FM) min^{-1}]	351.7±9.7a	370.5±2.3a	171.3±3.3d	145.0±1.4c	180.5±2.9c	150.8±2.9c	201.0±7.5b	190.5±5.7b

Zn-excess plants than in the Zn-deficient plants indicating vulnerability of their membranes to excess Zn in the medium.

Accumulation of soluble sugars, reducing sugars, and starch in the leaf and root tissues of Zn-optimum plants was higher than in Zn-deficient and Zn-excess plants (Table 2). However, considerable increment of phenols in leaves and roots was observed in the Zn-excess plants compared to the control (Table 2).

Although plants under both Zn-deficiency and Zn-excess had higher APX, CAT, and POD activities than control plants and leaves were more susceptible than the roots to Zn stress, the leaf tissues of Zn-excess plants exhibited the maximum activity of CAT. The activity of SOD in Zn-deficient plants was the superior among all treatments. Furthermore, it has been observed that the expression of *apx*, *cat*, and *sod* genes decreased with increasing Zn doses from deficiency to optimum (Fig. 2).

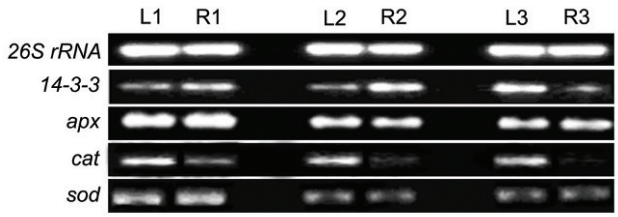

Fig. 2. RT-PCR analysis of *26SrRNA* (internal control), *14-3-3*, *apx*, *cat*, and *sod* transcripts in leaf (L) and root (R) of tea plants treated with 0 (L1, R1), 0.3 (L2, R2), and 3.0 μM (L3, R3) Zn for 8 weeks.

Discussion

Requirements of nutrients are specific for plants and any aberration can affect normal growth. Zn promotes growth when present at optimum concentrations but at higher levels, it inhibits the growth by interfering with the normal metabolic activities (Luo *et al*. 2010). Both deficient and surplus supply of Zn suppressed growth and reduced biomass production of tea plants. The growth of Zn-deprived plants became stunted because of impair-

ment in cell division. In *Hordeum vulgare*, dry mass of plants is also decreased under low Zn supply (Genc *et al*. 2002). Occurrence of chlorosis in tea leaves was in consonance with results described earlier in other plants (Bennet 1993). The development of Zn deficiency symptoms predominantly in the young leaves can be attributed to the poor phloem mobility of Zn (Marschner 1995). On the other hand, exposure of plants to excess of heavy metals may also induce reduction of growth, especially root growth (Weigel and Jager 1980) and decreased biomass production (Tewari *et al*. 2008). Surplus of Zn also acts upon root growth by inhibiting cell division (Luo *et al*. 2010). The results of the present study suggest that the biomass reduction depended upon the concentration of Zn and might be also a consequence of reduction in photosynthesis as well as enhanced ROS production. In Zn-deprived leaves, vacuoles were characteristic by electron dense material resembling phenolic compounds and phenol assay reaffirmed elevated content of phenolic compounds. The phenol accumulation in response to Zn-deficiency was also reported in pecan (Kim and Wetzstein 2003). Under high Zn, chloroplast damage, such as membrane rupture and thylakoid disintegration was noteworthy which would strongly influence the photosynthesis. Under heavy metal toxicity distortion of the thylakoid, chloroplast membrane and changes in the chloroplast ultrastructure were reported (Choudhury and Panda 2005) which correlates with the similar changes under Zn excess in tea. Under Zn-excess, mitochondria show swelling and cristae collapse in *Festuca rubra* (Davies *et al*. 1995) which are in consonance with the present report.

Zn deprivation and oversupply cause severe injuries to photosynthetic apparatus (Prasad and Strzalka 1999). RuBPC/O and many other chloroplastic and peroxisomal enzyme activities decrease under Zn-excess (Van Assche *et al*. 1980) which probably also inhibited rate of photosynthesis in the tea plantlets. In addition, g_s was significantly decreased in Zn-stressed (*i.e*., both deficient and excess) tea plants which reduced the P_N and E.

Content of Chl *a* and Chl *b* was decreased by deficient and excess supply of Zn whereas Car content only by Zn-excess. Reduction of pigments under Zn-deficiency was also observed in red cabbage, where Chl *a* and Chl *a*/*b* ratio decline (Hajiboland and Amirazad 2010). Similar decrease in Chl content under heavy metal stress was reported earlier in *Phaseolus vulgaris* (Siedlecka and Krupa 1996). The decline in Chl content in plants exposed to heavy metal stress is believed to be due to inhibition of Chl biosynthesis enzymes like δ-aminolaevulinic acid dehydratase (Padmaja *et al*. 1990) and protochloro-phyllide reductase (Van Assche and Clijsters 1990) as well as by substitution of Mg^{2+} by Zn^{2+} (Küpper *et al*. 1996). The increased Car/Chl ratio in Zn-deficient plants may be attributed to the adaptive response to elevated ROS generation because Car can scavenge ROS and protect Chl from photooxidation (Tewari *et al*. 2008).

Stimulation of ROS production and oxidative stress in tea under Zn stress was indicated by increased accumulation of O_2^-, H_2O_2, and MDA in the Zn-deficient and Zn-excess plants. Similar results were reported in mulberry under both deficiency and excess of Zn (Tewari *et al*. 2008) and also in citrus under B deficiency (Han *et al*. 2008). Membrane permeability is impaired in plants treated with Zn-deficiency (Grewal and Williams 1999) which is consistent with the idea that an alteration of plasma membrane lipids leads to an increase in permeability and impairment in plasma membrane proton gradient (Pinton *et al*. 1993).

Among antioxidative enzymes, APX activity is up-regulated under Zn-excess in mulberry (Tewari *et al*. 2008) and under Zn deficiency in red cabbage (Hajiboland and Amirazad 2010). Our finding corroborates with these previous reports. The increased activity of APX and amount of its transcripts in Zn stressed tea plants indicate the activation of ascorbate-glutathione cycle. In the tea plants, CAT activity was increased especially under Zn-excess and similar results were found in other plant species (Tewari *et al*. 2008, Luo *et al*. 2010, Hajiboland and Amirazad 2010). SOD was the first enzyme known to be reduced under Zn stress (Cakmak 2000). In wheat, the expression and activity of the Cu/Zn SOD and carbonic anhydrase are dependent on Zn-efficiency of individual genotypes (Hacisalihoglu *et al*. 2003). POD can be considered to be a useful marker for environmental stresses and increased POD activity under Zn-stress in the tea was in consonance with other reports (Tewari *et al*. 2008, Kösesakal and Ünal 2009).

In this study, reduction of sugar and starch content in the Zn-deficient and Zn-excess plants could be the consequence of impaired photosynthesis and modified sugar metabolism because Zn deficiency negatively influences the activity of aldolase and starch synthetase (Hajiboland and Amirazad 2010). Accumulation of free phenols under Zn-deficiency observed in the tea plants were also recorded in other plant species (Hajiboland and Amirazad 2010).

Some recent studies suggest that 14-3-3 proteins may play an important role in responses to mineral deficiencies (Wang *et al*. 2002). The activities of ion transporters are modulated by signaling proteins in response to environmental factors. Although the involvement of 14-3-3 has not been reported so far under Zn deficiency, the transcript abundance is regulated by potassium and iron deficiencies in tomato roots (Wang *et al*. 2002). Thus the similar hypothesis might hold true in our present finding where 14-3-3 transcript abundance was up-regulated under Zn stress.

In conclusion, exposure of tea cultivars to Zn-deficiency or Zn-surplus resulted in reduction of biomass, P_N, E and g_s but enhancement in ROS production, lipid peroxidation and membrane damage. Although Zn-stressed plants maintained high activities of antioxidant enzymes, their antioxidant system as a whole did not provide adequate protection against oxidative damage.

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