Growth, photosynthesis and antioxidant defense system in Zn-deficient red cabbage plants

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ABSTRACT

The effect of Zn deficiency was studied in red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*) plants grown in nutrient solution under controlled environmental conditions. Zinc starvation affected the number (61%), surface area (72%) and biomass (62%) of leaves more than root biomass (42%). Although chlorophyll fluorescence parameters revealed occurrence of photoinhibition following declined stomatal conductance and reduction of CO_2 available at carboxylation sites, photosynthesis apparatus was not damaged seriously under Zn deficiency conditions. Chlorophyll a, chlorophyll a/b ratio, soluble carbohydrates and starch declined but anthocyanins and free phenolics were accumulated under Zn deficiency conditions. Activity of ascorbate peroxidase, catalase and peroxidase enhanced under Zn deficiency conditions, whereas activity of superoxide dismutase declined in leaves but not in roots of Zn-deficient plants. Maintenance of superoxide dismutase activity and malondialdehyde content in roots demonstrated that roots were more protected against reactive oxygen species imbalance under Zn deficiency conditions compared with leaves that was correlated well with the lower sensitivity of roots to low Zn supply.

Keywords: antioxidant defense system; carbohydrates; chlorophyll fluorescence; CO_2 assimilation; *Brassica oleracea*; Zn deficiency

Zinc deficiency is a serious micro-nutritional disorder, threatening world food production. Zinc plays a fundamental role in several critical functions in the cell such as protein metabolism, gene expression, structural and functional integrity of biomembranes and photosynthetic carbon metabolism (Cakmak 2000). Some of metabolic changes brought about by Zn deficiency could be well explained by the function of Zn as a structural component of a special enzyme or involvement in specific steps in particular metabolic pathways (Marschner 1995). However, there are changes in the synthesis and metabolism of Zn-deficient plants that could not be explained directly by the presence of Zn in the metabolic pathway or enzyme structure. Such responses are regarded to be rather indirect effects of Zn deficiency.

Concerning the central role of Zn in stability of biomembranes and proteins (Cakmak 2000), Zn deficiency can affect the photochemical processes in the thylakoids, and thus inhibits biophysical processes of photosynthesis. The flow of electrons through PSII is indicative of the overall rate of photosynthesis and is an estimation of photosynthetic performance. Chlorophyll fluorescence measurements could be used to estimate the operating quantum efficiency of electron transport through PSII in leaves (Maxwell and Johnson 2000). Zinc deficiency can also cause a drastic decrease in chlorophyll content as well as a severe damage to the fine structure of chloroplasts (Chen et al. 2007).

In addition of the expected effect on photochemical processes, involvement of Zn in carbohydrate metabolism was demonstrated through its effect on net CO_2 assimilation rate via stomatal conductance (Sharma et al. 1995) and sugar transformations (Marschner 1995). Inhibition of activity of Rubisco, aldolase, sucrose synthase and starch synthetase in plant tissues due to Zn starvation has been suggested (Marschner 1995). Whether the effect of Zn nutritional status on stomatal conductance and starch or sucrose formation is a primary result of Zn deficiency still remains an open question. It was suggested that, Zn is involved in stomatal opening, possibly as a constituent of the enzyme carbonic anhydrase and/or as a factor in maintaining membrane integrity and K^+ uptake (Sharma et al. 1995). 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).

Oxygen radicals damage nucleic acids, proteins, chlorophyll and lipids, which leads to the formation of toxic products such as malondialdehyde (MDA) (Apel and Hirt 2004). Antioxidative enzymes such as ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and antioxidant compounds including ascorbate, glutathione as well as pigments such as anthocyanins and carotenoids play a key role in controlling the cellular level of these radicals and peroxides (Apel and Hirt 2004). Zinc is involved in the balance of ROS production and scavenging in plants because of its presence in the SOD, inhibitory effect on O_2^--producing NADH oxidase and protection of biomembranes via binding of Zn to SH-containing compounds (Marschner 1995).

Knowledge of functional role of Zn is incomplete and in some instance remains controversial. Although the effects of Zn on both photochemical and dark fixation processes in photosynthesis were documented, detailed information on the fundamental processes of energy absorption, utilization and dissipation of excess excitation energy by PSII under Zn starvation is still not available. On the other hand, effects of Zn deficiency on physiological processes are unlikely to be uniform for all plant species and/or all tissues.

Cruciferous vegetables act as a good source of natural antioxidants due to high levels of carotenoids, tocopherols and ascorbic acid. Red cabbage is particularly rich in anthocyanins with potent antioxidants and health promoting properties (Kaur and Kapoor 2001). Published works on the effect of Zn deprivation in hydroponically grown red cabbage plants are rare. The objective of this work was to study the effect of low Zn nutritional status on the photochemistry and CO_2 fixation of leaves, pigment content and activity of enzymes involved in the antioxidative defense system.

MATERIALS AND METHODS

Plant material and cultivation. Seeds of red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*) plants purchased from a commercial source, were surface-sterilized using sodium-hypochlorite at 5% and were germinated in the dark on filter paper soaked with saturated $CaSO_4$ solution. Eight-day-old seedlings were pre-cultivated for two weeks in 50% conventional Hoagland nutrient solution (Johnson et al. 1957) without Zn addition, then they were transferred to full strength chelator-buffered treatment solution. Chelator-buffered nutrient solution technique was used in order to reduce Zn contamination in the medium. Conventional nutrient solution, even without Zn addition, contained ~ 1.0μM Zn as contamination from water or chemicals, and plants in the –Zn medium did not show any growth difference compared to those in +Zn medium. The composition of chelator-buffered nutrient solution was similar with conventional Hoagland solution but contained HEDTA (N-2-hydroxyethyl-ethylenediamine-N,N',N'-triacetate) at 100μM to give a 50μM excess of HEDTA above the sum of the Cu, Fe and Mn concentrations. MES (2-[N-morpholino]-ethanesulfonate) was added at 2.0mM to buffer pH, which was adjusted at 6.0 using KOH. $ZnSO_4$ concentrations were 2.0 (low Zn) and 25 μM (adequate Zn), free Zn^{2+} activities were 32 and 725pM and calculated pZn^{2+}(–log[Zn^{2+}]) were 10.49 and 9.14, respectively. The chemical activity of Zn^{2+} and other ions in the nutrient solutions was calculated using the 2.0 version of GEOCHEM-PC.

Plants were grown 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^2s.

Plant harvest and analysis. Sixteen days after sowing, plants were harvested. Plants were divided into leaves and root, washed with double-distilled water and after blotting dry, fresh weight of samples was determined. After drying at 70°C for 2 days dry weight was estimated, then oven-dried samples were ashed in a muffle furnace at 550°C for 8 h, resolved in HCl and made up to volume by double-distilled water. Zinc concentration was determined by atomic absorption spectrophotometry (Shimadzu, AA 6300). Before harvest, chlorophyll (Chl) fluorescence and gas exchange parameters were determined. Another group of plants were harvested and used for pigment and carbohydrate analysis.

Determination of chlorophyll fluorescence and gas exchange parameters. Chlorophyll fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK). Measurements were carried out on the second youngest, fully expanded and attached leaf from 4 plants per treatment. 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) and F_v/F_0 ratios were recorded. Light-adapted leaves were used for measurement of initial (F_t) and maximum (F'_m) fluorescence. Calculations were made for F'_v =

$F'_m - F_t$, excitation capture efficiency of open PSII (F'_v/F'_m), F'_0 ($F'_0 = F_0/[(F_v/F_m) + (F_0/F'_m)]$), effective quantum yield of PSII ($\Phi_{PSII} = F'_m - F_t/F'_m$), photochemical quenching ($qP = F'_m - F_t/F'_m - F'_0$), non-photochemical quenching ($qN = 1-[(F'_m - F'_{0)}/(F_m - F_0)]$) and linear electron transport rate ($ETR = \Phi_{PSII} \times PFD \times 0.84 \times 0.5$, μmol/m^{2}s) (Maxwell and Johnson 2000).

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

Determination of chlorophyll, carotenoid, anthocyanin and total phenolic compounds concentrations. Leaf concentration of Chl a, b and carotenoid was determined according to Lichtentaler and Wellburn (1985) after extraction of pigments in cold acetone and keeping samples for 24 h in the dark at 4°C. 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 (Giusti and Wrolstad 2001). Concentration of total anthocyanins was expressed as g of cyanidine-3-glucoside/g of fresh weight. For determination of total phenolic compounds, leaves were extracted in 70% aqueous methanol and after centrifugation, supernatant was used for determination of phenolics using Folin-Ciocalteu reagent and gallic acid as standard (Swain and Hillis 1959).

Carbohydrates. Leaves were homogenized in 100mM 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 according to the method described by Magné et al. (2006).

Assay of antioxidant enzymes and concentration of metabolites. Activities of antioxidant enzymes and concentration of related metabolites were determined 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).

The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured by determining ascorbic acid oxidation, one unit of APX oxidizes ascorbic acid at the rate of 1 μmol/min at 25°C. Catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decrease in absorbance of H_2O_2 at 240 nm, unit activity was taken as the amount of enzyme, which decomposes 1 μmol of H_2O_2/min. Peroxidase (POD, EC 1.11.1.7) activity was assayed using the guaiacol test, the enzyme unit was calculated as enzyme protein required for the formation of 1 μmol tetraguaiacol/min. Total superoxide dismutase (SOD, EC 1.15.1.1) activity was determined using monoformazan formation test. One unit of SOD was defined as the amount of enzyme required to induce a 50% inhibition of NBT (nitroblue tetrazolium) reduction as measured at 560 nm, compared with control samples without enzyme aliquot. Soluble proteins were determined using a commercial Bradford reagent (Sigma) and BSA (bovine serum albumin) (Merck) as standard. Lipid peroxidation

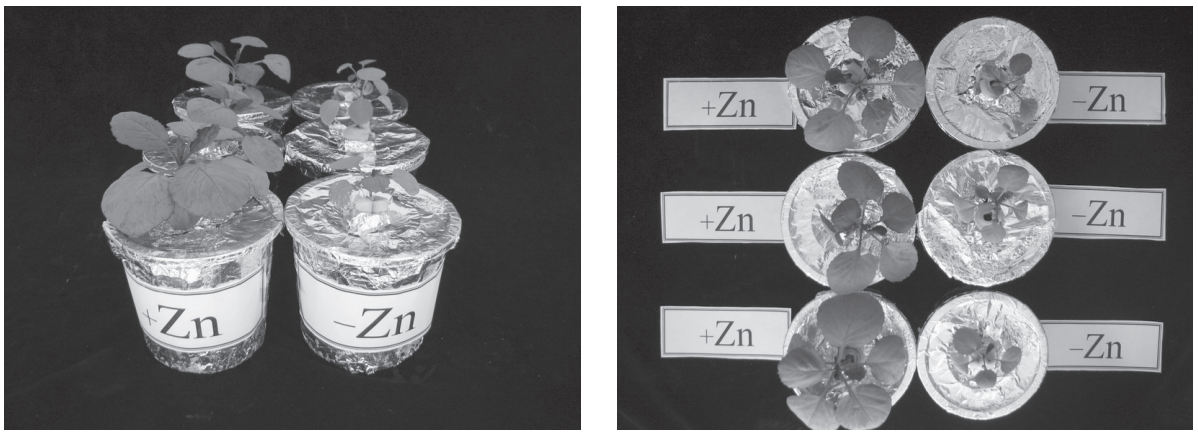

Figure 1. Red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown in hydroponic culture medium with adequate (+Zn) and low (–Zn) Zn supply

Table 1. Shoot and root dry weight (mg/plant) and root length (cm/plant) in red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply

Treatments	Shoot DW	Root DW	Root length
+Zn	1295 ± 88[a]	69 ± 6[a]	441 ± 15[a]
–Zn	491 ± 99[b]	40 ± 4[b]	216 ± 11[b]

The means refer to 4 repetitions ± SD. Data of each parameter followed by the same letter are not significantly different ($P < 0.05$)

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. The concentration of H_2O_2 was determined using potassium titanium-oxalate at 508 nm (Hajiboland and Hasani 2007).

Experiments were established 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

Zinc deprivation affected plant growth significantly (Figure 1). Shoot and root dry weight were depressed under low Zn supply up to 62% and 42% compared with control, respectively (Table 1). Zinc starvation affected the number and surface area of leaves more than shoot biomass. Zinc deficient plants had significantly less and smaller leaves, average number of leaves in control plants at harvest was 8.2 ± 0.75/plant, with a mean surface of 54 ± 8 cm^2/leaf, while Zn-deficient plants had on average 3.2 ± 0.92 leaves/plant with a surface area of 15 ± 3 cm^2/leaf. Root length was also affected significantly by low Zn supply by about 51% (Table 1).

Zinc concentration in leaves and roots was considerably lower in Zn-deficient plants compared with Zn-sufficient ones. At low supply, Zn content of leaves and roots were by 95% and 63% lower, respectively, compared with plants fed with adequate Zn. Reduction of Zn concentration in shoot and roots due to Zn deficiency was 86% and 36%, respectively (Figure 2).

Initial fluorescence (F_0) and maximum fluorescence (F_m) of dark-adapted leaves decreased significantly under low Zn supply. This reduction was 18% and 17% for F_0 and F_m, respectively, compared with control plants. In contrast to F_0 and F_m, optimal photochemical efficiency of PSII in dark-adapted leaves (F_v/F_m) and the proportion of active Chl associated with the reaction centers (RCs) of PSII (F_v/F_0) remained unchanged. In addition, the excitation capture efficiency of open PSII RCs (F'_v/F'_m) rather increased in Zn-starved plants. Quantum yield of PSII (ΦPSII) and electron transfer rate (*ETR*) were not influenced by Zn deficiency conditions. However, the amount of oxidized PSII reaction centers ready for reduction, i.e. photochemical quenching (*qP*), that reflects the capacity to utilize absorbed energy through metabolism and growth, decreased significantly in Zn-deficient leaves. The non-photochemical quenching (*qN*) that reflects the capacity to dissipate excess absorbed energy as heat was also decreased by low Zn supply (Table 2).

Net CO_2 assimilation rate (*A*) of Zn-deficient plants was by 21% lower than control leaves. Transpiration rate and stomatal conductance were also lower by about 30% and 40% in plants grown under low Zn supply compared with con-

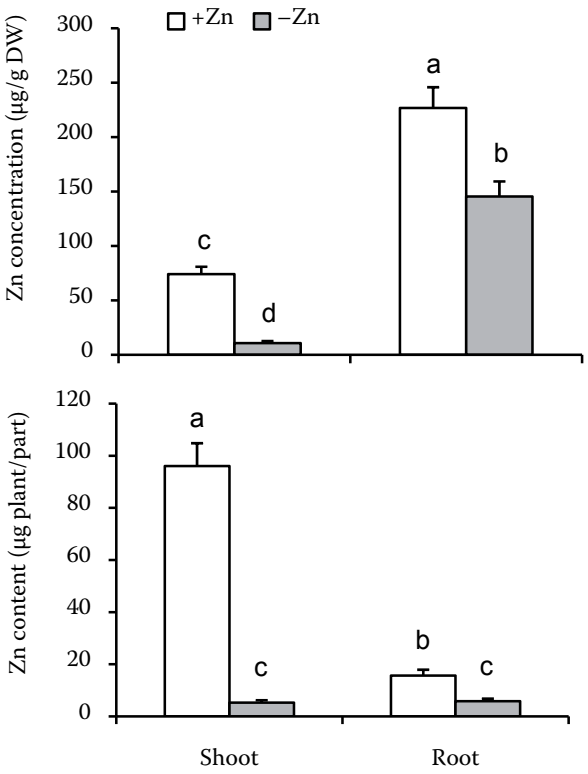

Figure 2. Zn concentration (µg/g DW) and content (µg/plant) in red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply. The means refer to 4 repetitions ± SD. Bars indicated by the same letter are not significantly different ($P < 0.05$)

Table 2. Chlorophyll fluorescence parameters including F_0 (initial flourescence), F_m (maximum fluorescence), F_v/F_m (photochemical efficiency of PSII), F_v/F_0 (ratio of variable to initial fluorescence), F'_v/F'_m (excitation capture efficiency of open PSII), q_P (photochemical quenching), q_N (non-photochemical quenching), Φ_{PSII} (quantum yield of PSII) and ETR (electron transport rate) in the leaves of red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply for two months

Treatments	F_0	F_m	F_v/F_m
+Zn	582 ± 11[a]	3499 ± 230[a]	0.833 ± 0.01[a]
–Zn	475 ± 15[b]	2901 ± 204[b]	0.836 ± 0.01[a]
	F_v/F_0	F'_v/F'_m	q_P
+Zn	5.01 ± 0.31[a]	0.774 ± 0.012[b]	0.996 ± 0.020[a]
–Zn	5.09 ± 0.24[a]	0.815 ± 0.011[a]	0.858 ± 0.024[b]
	q_N	Φ_{PSII}	ETR
+Zn	0.360 ± 0.092[a]	0.771 ± 0.011[a]	129 ± 1.9[a]
–Zn	0.147 ± 0.083[b]	0.781 ± 0.010[a]	131 ± 1.7[a]

The means refer to 4 repetitions ± SD. Data of each parameter followed by the same letter are not significantly different ($P < 0.05$)

trol, respectively. The molar ratio of intercellular to atmospheric CO_2 (*Ci*/*Ca*) was not affected by Zn nutritional status of plants (Table 3).

With the exception of SOD, activity of three other studied antioxidant enzymes including APX, CAT and POD increased significantly by low Zn supply both in leaves and roots. Activity of APX in low Zn roots was 3.2 times greater than control. This change was more pronounced than that in shoot with 84% increase under Zn deficiency conditions. For POD, in contrast, leaves responded more strongly than roots with 2.3 times higher activity compared with control. The enhancement of CAT activity due to low Zn supply was similar for shoot and root and reached up to 115%. As expected, activity of SOD decreased significantly up to 28% in leaves due to Zn deficiency but was not affected in root. Accumulation of H_2O_2 and MDA was higher in Zn-deficient plants compared with control, with the exception of roots showing no significant influence of Zn nutrition on MDA content (Table 4).

Zinc deficiency conditions had no influence on the concentration of Chl b and carotenoids. In contrast, Zn-deficient leaves showed a significant reduction of chlorophyll a/b ratio by about 21% because of a strong negative effect on Chl a content. Anthocyanins and soluble phenolics were accumulated in Zn-deficient leaves up to 58% and 29% compared with control, respectively (Table 5).

Leaf content of both total soluble sugars and starch decreased under low Zn supply. In shoot, Zn deficiency conditions caused reduction of soluble sugars and starch up to 27% and 15%, respectively, compared with control. The corresponding values for root were 16% and 55% (Figure 3).

DISCUSSION

The most obvious symptoms of Zn deficiency in red cabbage plants were stunted growth because of shortening of petioles, drastic decrease in leaf surface area (up to 72%) and number of leaves (61% reduction). Strong reduction of leaf size and number indicated a critical role for Zn in both cell expansion and division. Growth of plants that left to grow for further 14 days under Zn deficiency conditions was dramatically inhibited and no more

Table 3. Gas exchange parameters including net photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance to water vapor (g_s) and the ratio of intercellular air space and atmospheric CO_2 molar fractions (*Ci/Ca*) in red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply for two months

Treatments	*A* (μmol/m^2/s)	*E* (mmol/m^2/s)	g_s (mmol/m^2/s)	*Ci/Ca*
+Zn	9.28 ± 1.01[a]	4.12 ± 0.98[a]	0.48 ± 0.03[a]	1.06 ± 0.93[a]
–Zn	7.32 ± 0.76[b]	2.89 ± 0.60[b]	0.29 ± 0.00[b]	1.03 ± 0.35[a]

The means refer to 4 repetitions ± SD. Data of each column followed by the same letter are not significantly different ($P < 0.05$)

Table 4. Specific activity of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and concentration of malondialdehyde (MDA) and hydrogen peroxide in the shoot and roots of red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply for two months

	Treatments	Shoot	Root
APX (μmol/mg protein/min)	+Zn	6.3 ± 0.9^{c}	2.8 ± 0.5^{d}
	–Zn	11.6 ± 0.4^{a}	8.8 ± 0.6^{b}
CAT (μmol/mg protein/min)	+Zn	115 ± 21^{bc}	73 ± 3^{c}
	–Zn	252 ± 41^{a}	157 ± 6^{b}
POD (μmol/mg protein/min)	+Zn	10.2 ± 0.2^{c}	10.2 ± 0.3^{c}
	–Zn	23.7 ± 0.3^{a}	19.6 ± 0.4^{b}
SOD (U/mg protein)	+Zn	425 ± 62^{a}	502 ± 55^{a}
	–Zn	305 ± 6^{b}	505 ± 18^{a}
MDA (nmol/g FW)	+Zn	5.2 ± 0.5^{b}	1.4 ± 1.1^{a}
	–Zn	9.3 ± 2.2^{a}	1.5 ± 1.0^{a}
H_2O_2 (μmol/g FW)	+Zn	5.5 ± 1.2^{b}	11.8 ± 0.7^{b}
	–Zn	11.4 ± 0.9^{a}	14.4 ± 0.6^{a}

The means refer to 4 repetitions ± SD. Data of each enzyme or metabolite followed by the same letter are not significantly different ($P < 0.05$)

new leaf emerged (data not shown). Lower leaf surface area could result both from impaired cell division and cell expansion. The presence of enough Zn in meristematic tissues is necessary because of its function in DNA metabolism (Marschner 1995). Impairment of cell expansion due to Zn deficiency resulted likely from decreased accumulation of K^+ and other solutes in leaf cells following loss of membrane integrity as reported for guard cells in Zn-deficient leaves (Sharma et al. 1995).

In contrast to graminaceous species such as wheat (Cakmak and Marschner 1996) and rice (Hajiboland and Beiramzadeh 2008), visual leaf symptoms such as chlorosis or necrosis were not observed in Zn-deficient red cabbage plants with the exception of slightly darker red color due to increased anthocyanin content. Symptoms of chlorosis, necrosis or discoloration in leaves of Zn-deficient cereal species that are not accompanied with marked change in leaf size, could be regarded secondary effects caused by toxicity of phosphorus, boron or photooxidation (Marschner 1995). However, 'little leaf' and 'rosetting' symptoms observed in this work in red cabbage plants similar with other dicotyledons are primary effects of the absence of Zn on impairment of cell division in meristems as well as cell expansion in developing leaves.

Zinc content (μg Zn/plant part) of roots and particularly leaves declined strongly under low supply of Zn. Similarly, Zn concentration (μg Zn/g dry weight)

Table 5. Concentration of chlorophyll a, b (mg/g FW), the ratio of chlorophyll a/b and concentration of anthocyanins (mg cyaniding-3-glucosid/g FW), carotenoids (mg/g FW) and total soluble phenolics (mg gallic acid/g FW) in leaves of red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply for two months

Treatments	Chl a	Chl b	Chl a/b
+Zn	2.08 ± 0.06^{a}	1.35 ± 0.04^{a}	1.55 ± 0.08^{a}
–Zn	1.59 ± 0.03^{b}	1.30 ± 0.02^{a}	1.22 ± 0.04^{b}
	carotenoids	anthocyanins	phenolics
+Zn	0.81 ± 0.02^{a}	1.9 ± 0.1^{b}	3.68 ± 0.19^{b}
–Zn	0.75 ± 0.02^{a}	3.0 ± 0.1^{a}	4.73 ± 0.26^{a}

The means refer to 4 repetitions ± SD. Data of each column followed by the same letter are not significantly different ($P < 0.05$)

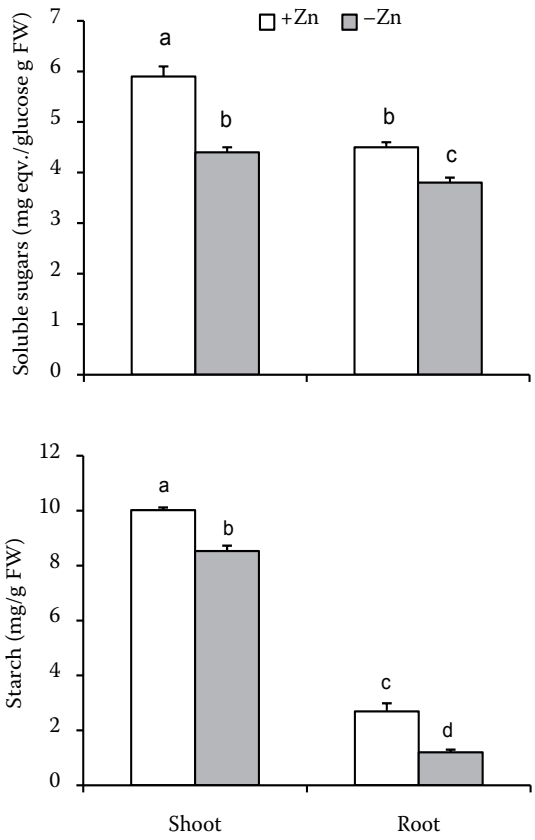

Figure 3. Total soluble sugars (mg eqv. glucose/g FW) and starch (mg/g FW) content of leaves and roots in red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply. The means refer to 4 repetitions ± SD. Bars indicated by the same letter are not significantly different ($P < 0.05$)

decreased under deficiency conditions and fell below 11 μg Zn/g dry weight for leaves. The critical Zn deficiency level, if 90% of the maximal shoot dry weight is used as reference point is about 15–20 μg Zn/g dry weight for soil-grown plants (Marschner 1995) and for red cabbage in this work it could be estimated as 64 μg Zn/g dry weight for leaves. Higher amounts obtained in our experiment could be related to differences among species in the capacity to take up Zn or differences between hydroponic and soil conditions (Marschner 1995).

The initial Chl fluorescence yield (F_0) that reflects the minimal fluorescence yield when all Q_A are in oxidized state, reduced in Zn-deficient leaves. In addition, Zn-deficient leaves had significantly smaller maximal fluorescence yield (F_m) value compared to the control demonstrating likely a diminished pool of PQ (Ouzounidou et al. 2003). The preservation of F_v/F_m and F_v/F_0 and an increase of F'_v/F'_m indicated that the thylakoid constituents were not damaged seriously in Zn-deprived leaves. It was suggested that, for nutrients without direct involvement in the electron transport or Chl synthesis such as Zn, a close linkage between nutritional status of leaves and spectral characteristics seems unlikely (Adams et al. 2000). However, there are reports on significant reduction of maximum quantum efficiency of PSII (Wang and Jin 2005) and severe damage to the ultrastructure of chloroplasts in plants subjected to inadequate Zn supply (Chen et al. 2007). We observed also that in contrast to well-watered plants, Zn deficiency caused a serious damage to photosynthetic apparatus in the leaves of Zn-deficient red cabbage plants subjected to drought stress (Hajiboland and Amirazad 2010).

Reduction of photochemical quenching could be related to photoinhibition rather than to a direct damage of PSII (Baker and Bowyer 1994). One of the causes of photoinhibition could be lower Chl content in Zn-deficient leaves. Leaf Chl concentration has a crucial role for the susceptibility to photoinhibition and leaves with less Chl are more susceptible to photoinhibition (Pätsikkä et al. 2002). Moreover, Zn deficiency conditions likely cause an excess of reducing power ($NADP + H^+$) because of reduction in the CO_2 available at carboxylation sites i.e. stomatal closure (Harbinson 1994). In addition, simultaneous reduction of qP and qN indicates an overexcitation of the photochemical system likely accompanied by accumulation of reduced electron acceptors. Under such conditions the probability of generation of reactive radicals which further injure PSII components is very high (Barber and Anderson 1992). With more severe Zn deficiency or longer growth period under deficiency conditions serious damage to photosystems was expected to occur in Zn-deficient leaves.

A comparison of the ratio of Chl a/b revealed a preferential damage of Chl a in Zn-deficient leaves, however, changes in this ratio were not reflected in the Chl a fluorescence parameters such as F_v/F_0. Total carotenoid pool was not affected by low Zn. Yet, the Chl/carotenoid ratio decreased in Zn-deficient leaves that could be the reason for lower efficiency of leaves in heat dissipation reflected in lower qN in Zn-deficient leaves.

Net CO_2 uptake per leaf surface area was depressed by low Zn supply. In addition, the remarkable reduction of total plant leaf area likely affected whole plant photosynthesis and contributed further to the low biomass production under Zn deficiency conditions. Stomatal conductance was significantly lower in Zn-deficient leaves, which caused in turn lower transpiration. Reduction of stomatal conductance due to low

supply of Zn was reported for other plants such as maize (Wang and Jin 2005) and rice (Hajiboland and Beiramzadeh 2008). Involvement of Zn in stomatal opening was attributed to the structural role of Zn in carbonic anhydrase needed for maintaining adequate HCO_3^- in the guard cells and also to controlling effect of Zn on K^+ uptake by the guard cells (Sharma et al. 1995). However, since stomatal aperture is affected also by deficiency of other nutrients such as Fe (Molassiotis et al. 2006; Hajiboland and Beiramzadeh 2008), it seems to be influenced rather indirectly by factors being common under deficiency of other nutrients, e.g. loss of membrane integrity and passive leakage of K^+ from guard cells.

The exact role of Zn in photosynthesis is obscure and is complicated by the multiple effects of Zn deficiency on processes closely related to photosynthesis and carbon fixation. Reports on the effect of Zn deficiency on the content of sucrose and starch are controversial, a decline in the level of sucrose under Zn deficiency was reported for sugar beet and maize plants, while in bean, cabbage and peanut Zn deficiency increased the concentration of sucrose or reduced sugars (Brown et al. 1993). Starch content of leaves declined in peanut, while increased in cabbage and bean due to Zn deficiency (Brown et al. 1993). In our work, reduction of soluble and insoluble (starch) carbohydrates could be the result of impaired leaf photosynthesis as described above, as well as of modified sugar metabolism. Zinc deficiency greatly depresses the activity of aldolase and starch synthetase (Marschner 1995). Altered distribution of carbohydrates between leaves and roots which was attributed to impaired sucrose loading from source leaves (Marschner and Chamak 1989) was not observed in our experiment because of similar partitioning of carbohydrates between leaves and roots under adequate and low Zn supply. In addition, a clear reduction of both soluble and non-soluble carbohydrates in leaves and roots suggested that, in contrast to other reports (Marschner 1995), in our work it was the lower supply of photoassimilates that limited strongly plant growth under Zn deficiency conditions.

Zinc-deficient red cabbage leaves accumulated more anthocyanins than control ones. Accumulation of anthocyanins was observed in cereal leaves such as sorghum (Furlani et al. 1986) and rice (Hajiboland and Beiramzadeh 2008) under low Zn supply. Although antioxidative effect of anthocyanins was demonstrated in experiments involving rats or animal cell cultures, a growing body of experimental evidence does indeed indicate that anthocyanins contribute to the control of levels of ROS in plant cells (Anderson and Jordheim 2005). Anthocyanins are synthesized in the cytoplasm as colorless tautomers and both the cytosolic and vacuolar red forms of anthocyanins have strong antioxidant potential in plant cells (Anderson and Jordheim 2005). Under Zn deficiency conditions that caused imbalance between production and scavenging ROS, anthocyanins contribute likely to the alleviation of oxidative stress. Reduction of *qN* in Zn -deficient leaves indicated lower efficiency of photo-protective mechanisms through carotenoids. Probably, anthocyanins protect the photosystems from damaging effects under these conditions.

Regarding involvement of Zn in the balance between production and scavenging of ROS in plants (Marschner 1995), Zn deficiency affects ROS metabolism more than deficiency of other micronutrients. In our work, activity of all antioxidant enzymes with the exception of SOD increased under low Zn supply. Decreased total SOD activity in plants exposed to Zn deficiency demonstrated that eventual induction of SOD species that do not require Zn (Alscher et al. 1997) was not able to compensate strong depression of Cu/ZnSOD activity. SOD activity was suggested to be an indicator of Zn nutritional status of plants and is the first enzyme activity known to be reduced under low Zn stress (Cakmak 2000). A significant increase in H_2O_2 content though an increase in the activity of H_2O_2 scavenging enzymes in Zn-deficient plants suggested an imbalance between production and scavenging H_2O_2. The increase in H_2O_2 concentration may be attributed to ROS generating activity of redox active Fe in the Zn-deficient tissues (Cakmak 2000).

A clear difference was observed between roots and leaves in their antioxidant defense system. In contrast to leaves, SOD activity was not affected in roots of Zn-deficient plants. Similarly, MDA was not accumulated in roots of Zn-deficient plants and increase in the root concentration of H_2O_2, because the Zn deficiency was only 22% compared with leaves with up to 107% enhancement. It could be suggested that an imbalance between production and scavenging of ROS under Zn deficiency conditions was greater for leaves than roots. It is likely that, leaves were exposed to greater oxidative stress than roots because of production of more ROS during light reactions, greater reduction of SOD and further accumulation of MDA and H_2O_2. Differences between leaves and roots in the balance of ROS were correlated with different growth response of these organs to low Zn, indicating that oxidative damage is the basis of disturbance in plant growth caused by Zn deficiency (Cakmak 2000).

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