Drought tolerance in Zn-deficient red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*) plants

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Abstract

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Effects of Zn deficiency were studied in red cabbage plants under well-watered or drought conditions. Impairment of growth due to Zn deficiency was higher under drought compared with well-watered conditions. Drought stress caused a drastic decline in Zn content and led to a damage of photosynthetic apparatus in Zn-deficient but not Zn-sufficient leaves. Net assimilation and transpiration rate were strongly reduced under Zn deficiency and drought conditions following reduction of stomatal conductance. Activity of antioxidant enzymes, with the exception of superoxide dismutase, increased under Zn deficiency conditions, while drought enhanced activity of all studied enzymes concomitant with accumulation of malondialdehyde and H_2O_2. The intensifying effect of drought on Zn-deficient leaves could be explained by impaired leaf photochemical events, reduction of whole plant photosynthesis and imbalance between production and scavenging of reactive oxygen species. Water use efficiency, water and osmotic potential of drought-stressed plants were higher under low compared with adequate Zn supply, however, these parameters were not critical for plant growth response under combinative effect of drought and Zn deficiency.

Keywords: Zn deficiency; drought stress; antioxidant defense system; red cabbage; chlorophyll fluorescence; CO_2 assimilation

Zinc deficiency is a widespread micronutrient deficiency particularly in arid and semi-arid regions (Cakmak et al. 1996). Zinc plays a fundamental role in protein metabolism, gene expression, biomembranes integrity 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 enzymes or involvement in particular metabolic pathways (Marschner 1995). However, there are changes in the synthesis and metabolism that could be regarded as indirect effects of Zn deficiency.

Chlorophyll (Chl) fluorescence measurements could be used to assess the change in the function of PS II under different environmental conditions (Maxwell, Johnson 2000). Effect of Zn deficiency on photochemical processes as well as CO_2 assimilation rate was reported for some plant species (Hajiboland, Beiramzadeh 2008; Wang et al. 2009). These effects are likely mediated by changes in the ultrastructure of thylakoid membranes (Chen et al. 2007), in stomatal conductance (Sharma et al. 1995) and carbohydrate metabolism (Marschner 1995).

Oxidative stress occurs when there is a serious imbalance between the production of reactive oxygen species (ROS) and antioxidant defense capacity. Antioxidative enzymes such as ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) play a key role in controlling the cellular level of these radicals and peroxides

(Apel, 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). Stress factors, such as drought, increase the formation of ROS causing damage to proteins, membrane lipids and cellular components (Apel, Hirt 2004). Drought inhibits photochemical events (Bruce et al. 2002) and decreases the activity of enzymes in Calvin cycle.

Nutritional status of plants has a great influence on the tolerance of plants to environmental stresses such as drought, salinity and high light intensity (Marschner 1995). Because of a wide spectrum of Zn deficiency effects on plants metabolism, particularly in the balance between ROS production and scavenging, it is expected that Zn deficiency would affect drought tolerance of plants and the extent of growth impairment by drought would be influenced greatly by their Zn nutritional status. Reports on the effect of Zn nutritional status on drought tolerance of plants are controversial. Growth and net photosynthesis rate of drought-stressed maize plants were not improved by Zn supply (Wang et al. 2009). In contrast, sensitivity to Zn deficiency stress became more pronounced when durum wheat plants were drought-stressed (Bađci et al. 2007). No information is available on the Zn uptake and transport in plants subjected to water deficit.

Effects of Zn deficiency on physiological processes are unlikely to be uniform for all plant species and/or all tissues. Red cabbage is one of cruciferous vegetables particularly rich in anthocyanins with potent antioxidant and health promoting properties (Kaur, Kapoor 2001). Published works on the effect of Zn deprivation in drought tolerance of plants in general and red cabbage in particular are rare. The objective of this work was to study the effect of low Zn nutritional status on the responses of red cabbage plants to drought stress. In addition of photochemistry and CO_2 fixation, antioxidant defense system was studied in Zn deficient plants subjected to drought stress.

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%, then they were germinated in the dark on filter paper soaked with saturated $CaSO_4$ solution. Eight-day-old young seedlings were transferred to the pots filled with acid-washed, sieved perlite (1–2 mm particles) irrigated for two weeks with water or 50% conventional modified Hoagland nutrient solution (Johnson et al. 1957) without Zn at field capacity. Thereafter, plants were irrigated with water or full strength chelator-buffered nutrient solution. The composition of chelator-buffered nutrient solution was similar with conventional Hoagland solution but contained 100μM HEDTA (N-2-hydroxyethyl-ethylenediamine-N,N',N'-triacetate) and 2mM MES (2-[N-morpholino]-ethanesulfonate) at pH 6.0 using KOH. $ZnSO_4$ concentrations were 2.0μM (low Zn) and 25μM (adequate Zn), free Zn^{2+} activities were 32pM 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 version 2.0 of GEOCHEM-PC (Parker et al. 1995). Plants were irrigated either at 100% (control) or 30% field capacity (drought treatment) by daily weighing. Because the used pots had no drainage hole, cares were taken to avoid accumulation of salts in the substrate. The volume of full strength nutrient solution used for irrigation was started with 200 ml per plant per week in the earlier growth phase and increased gradually and reached up to 500 ml at the final phase of growth.

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

After growing for 50 days (72 days after sowing), plants were harvested. Leaves were removed from the base of the rosettes and roots were separated from perlite particles carefully, washed with double-distilled water and after blotting dry, fresh weight and root length (Tennant 1975) were determined. After drying at 70°C for 2 days to determine dry weight, 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 6500).

Before harvest, chlorophyll fluorescence and gas exchange parameters were determined. Another group of plants were harvested and used for assay of enzymes, pigments and metabolites.

Determination of chlorophyll fluorescence and gas exchange parameters

Chlorophyll fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK) for both dark-adapted and light-adapted leaves. Measurements were carried out on the second youngest, fully expanded and attached leaf from four 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 PS II (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 PS II (F'_v/F'_m), F'_0 ($F'_0 = F_0/[(F_v/F_m) + (F_0/F'_m)]$) (Oxborough 2004), effective quantum yield of PS II ($\Phi_{PS\,II} = F'_m - F_t/F'_m$) (Genty et al. 1989), 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 ($ETR = \Phi_{PS\,II} \times$ Photon Flux Density $\times 0.84 \times 0.5$, μmol/m^{2}s) (Krall, Edwards 1992).

CO_2 assimilation and transpiration rates were measured in parallel for chlorophyll 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 intensity at the leaf surface (Q_{leaf}) of 200, 300, 400, and 500 μmol/m^{2}s. Detailed results of gas exchange parameters were reported only for measurements under 400 μmol/m^{2}s i.e. similar light conditions with growth chamber. The net photosynthesis rate by unit of leaf area (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, mol/m^{2}s) were calculated using the values of CO_2 and humidity variation inside the chamber. The photosynthetic water use efficiency (*WUE*) was determined by the ratio of net photosynthesis rate (A) to transpiration rate (E) ($WUE = A/E$).

Determination of chlorophyll a and b

Leaf concentration of chlorophyll a, b and carotenoids were determined according to Lichtenthaller and Wellburn (1985) after extraction of pigments in cold acetone and allowing the samples to stand for 24 h in the dark at 4°C.

Carbohydrates

Leaves were homogenized in 100mM phosphate buffer (pH 7.5) at 4°C, after centrifugation at 12,000 g for 15 min, supernatants were used for total soluble sugars determination, whereas the pellets were kept for starch analysis according to the method described in Magné et al. (2006).

Assay of antioxidant enzymes and concentration of metabolites

Activity of antioxidant enzymes and concentration of related metabolites were undertaken according to optimized protocols described elsewhere (Hajiboland, 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 nitro blue tetrazolium (NBT) reduction as measured at 560 nm, compared with control samples without enzyme aliquot. Soluble proteins were determined using a commercial Bradford reagent (Sigma) and bovine serum albumin (BSA) (Merck) as standard. Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing

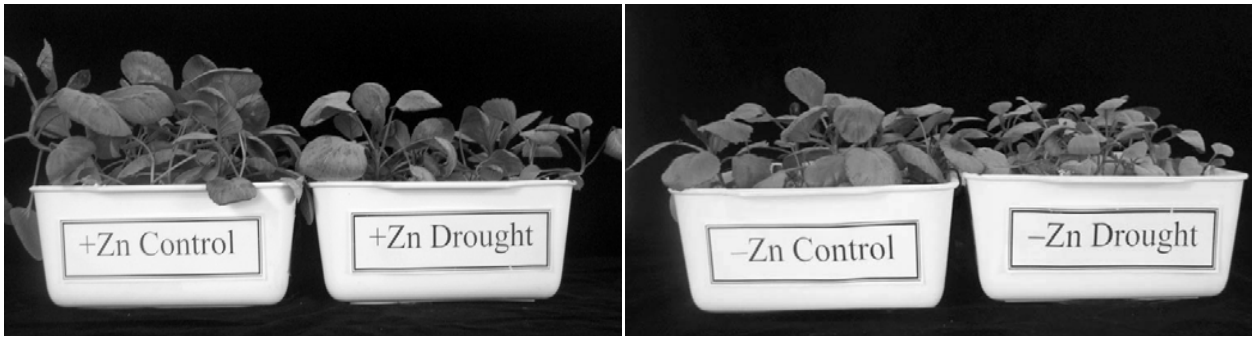

Fig. 1 Effect of low Zn supply on growth of red cabbage plants under well watered (control) and drought conditions

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. Proline was extracted in 3% sulfosalicylic acid and after centrifugation the supernatant was treated with acetic acid and acid ninhydrin and boiled for 1 h. The absorbance of samples was determined at 520 nm and compared with standard curve created for proline (Sigma) (Hajiboland, Hasani 2007).

Leaf water and osmotic potential

Leaf osmotic potential (ψ_π) and water potential (ψ_w) was determined in the second youngest leaf harvested at 1 h after light on in the growth chamber. Leaves were homogenized in pre-chilled mortar and pestle and centrifuged at 4,000 g for 20 min at 4°C. The osmotic pressure of samples was measured by an osmometer (Micro-Osmometer, Heman Roebling MESSTECHNIK, Germany), and the miliosmol data were recalculated to MPa. Water potential was measured using a pressure chamber (DTK-7000, Japan).

Experiments were undertaken in a complete randomized block design with 4 replications. Statistical analyses were carried out using SigmaStat (3.02) with Tukey test ($P < 0.05$).

RESULTS

Shoot and root dry weight and root length depressed under low Zn supply and drought stress (Fig. 1). The effect of Zn deficiency was more pronounced in shoot than root under both watering regimes. In plants subjected to low Zn supply, both shoot and root dry weight (but not root length) were decreased further by drought stress, leading to the lowest amounts under dual effect of low Zn and drought. Reduction of shoot biomass due to low Zn supply was 62% and 70% under well-watered and drought stress conditions, respectively; the corresponding values for root biomass were 42% and 46%. These data suggested that shoots are more susceptible to Zn deficiency compared with roots and drought stress intensifies the effect of low Zn on plant dry matter production (Fig. 2).

Chlorophyll a and b contents were influenced negatively by both low Zn supply and drought stress. Reduction of Chl a due to low Zn supply was greater compared with Chl b, leading to a significant decline of Chl a/b ratio in Zn-deficient leaves. However, Chl a/b ratio was not influenced by drought stress, which indicated that water deficit induced destruction of Chl a and Chl b with similar extent (Fig. 2).

Zinc content was affected by both Zn supply level and drought stress slightly or significantly. Zinc content of leaves, particularly in well-watered plants, was declined dramatically (93%) under deficiency conditions. Drought stress also strongly affected Zn content of leaves; it reached up to 89% in Zn-sufficient plants. In contrast, root Zn content responded only slightly to both Zn supply levels and drought conditions. Reduction of Zn content due to low Zn supply in root was significant only in well watered plants (35%) and the effect of drought conditions was not significant either at low or adequate Zn supply. Apart from the difference between leaves and roots, plant total Zn content, i.e. the height of histograms, was drastically reduced under both Zn deficiency and drought conditions (Fig. 3).

Leaves of Zn-deficient plants had significantly lower initial fluorescence (F_0) and maximum fluorescence (F_m). F_v/F_m ratio was decreased due to low Zn supply only under drought conditions. In con-

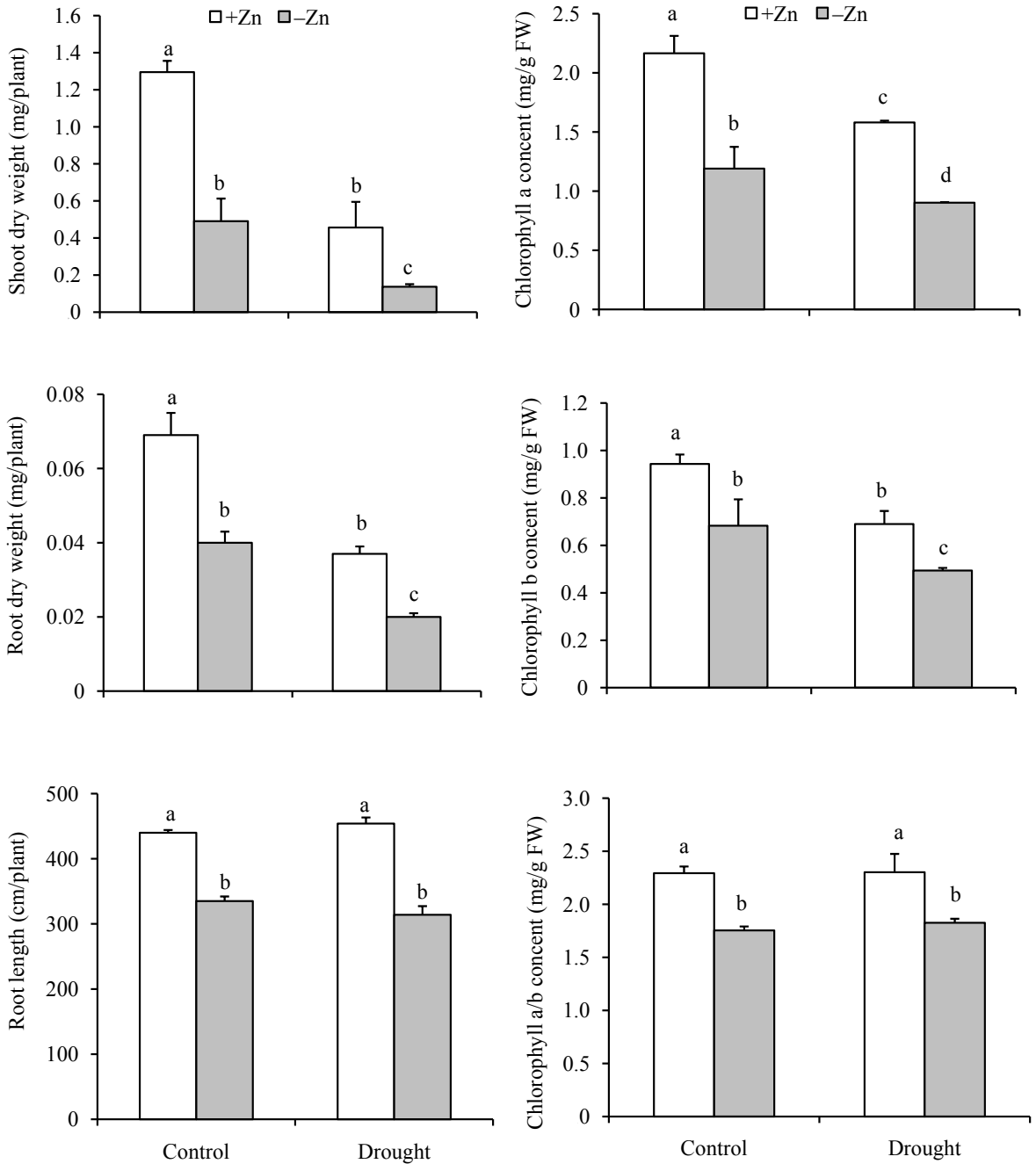

Fig. 2. Shoot and root dry weight (mg/plant), root length (cm/plant), leaf chlorophyll a and b content (mg/g FW) and the ratio of chlorophyll a/b in red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply under control and drought conditions. The means refer to 4 repetitions ± SD. Bars indicated by the same letter are not significantly different ($P < 0.05$)

trast, the ratio of F_v/F_0 was affected neither under control nor under water deficit. In addition, the excitation capture efficiency of open PS II RCs (F'_v/F'_m) was rather increased in Zn-starved plants irrespective of the watering conditions. Quantum yield of PS II ($\Phi_{PS\ II}$) and electron transfer rate (ETR) were not influenced by Zn deficiency conditions, either. However, the amount of oxidized PS II 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

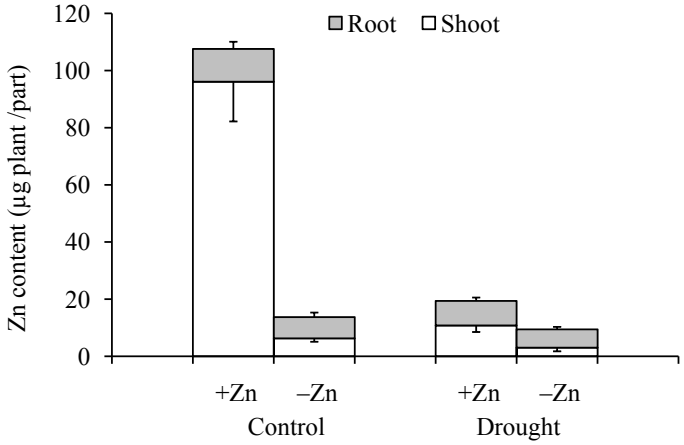

Fig. 3. Content (µg/plant part) of Zn in shoot and root in red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply under control and drought conditions. The means refer to 4 repetitions ± SD. Bars indicated by the same letter are not significantly different ($P < 0.05$)

as heat was also depressed by low Zn supply. The highest amounts for $\Phi_{PS\ II}$ and ETR were achieved in plants fed with adequate Zn but suffered from drought stress. Only in drought-stressed plants, low Zn supply caused a significant reduction of $\Phi_{PS\ II}$ and ETR (Table 1).

Net assimilation rate (A) was affected both by Zn deficiency and drought conditions. In well watered plants, Zn starvation affected net photosynthesis rate up to 40%, but in drought-stressed plants low Zn supply changed the net photosynthesis rate only up to 24%. Drought stress depressed CO_2 assimilation rate in Zn-sufficient plants up to 53%, this reduction for Zn-deficient plants was 40%. Transpiration rate (E) was declined both by low Zn supply and drought stress, significantly or in tendency. Similar to pho-

Table 1. Chlorophyll fluorescence parameters including F_0 (initial flourescence), F_m (maximum fluorescence), F_v/F_m (photochemical efficiency of PS II), F_v/F_0 (ratio of variable to initial flourescence), F'_v/F'_m (excitation capture of open PS II), qP (photochemical quenching), qN (non-photochemical quenching), $\Phi_{PS\ II}$ (quantum yield of PS II), and ETR (electron transport rate) in leaves of red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months with adequate (+Zn) and low (–Zn) Zn supply under control or drought conditions

Treatments		F_0	F_m	F_v/F_m
Control	+Zn	582 ± 11[a]	3499 ± 230[a]	0.833 ± 0.01[a]
	–Zn	475 ± 15[c]	2901 ± 204[b]	0.836 ± 0.01[a]
Drought	+Zn	523 ± 5[b]	3317 ± 111[a]	0.842 ± 0.00[a]
	–Zn	414 ± 12[d]	2704 ± 200[b]	0.772 ± 0.01[b]
		F_v/F_0	F'_v/F'_m	qP
Control	+Zn	5.01 ± 0.31[a]	0.541 ± 0.017[b]	1.425 ± 0.047[a]
	–Zn	5.09 ± 0.24[a]	0.604 ± 0.018[a]	1.295 ± 0.054[b]
Drought	+Zn	5.34 ± 0.22[a]	0.532 ± 0.006[b]	1.431 ± 0.014[a]
	–Zn	5.05 ± 0.12[a]	0.599 ± 0.028[a]	1.284 ± 0.065[b]
		qN	$\Phi_{PS\ II}$	ETR
Control	+Zn	0.846 ± 0.025[a]	0.771 ± 0.011[b]	129 ± 1.9[b]
	–Zn	0.782 ± 0.033[b]	0.781 ± 0.010[b]	131 ± 1.7[b]
Drought	+Zn	0.839 ± 0.006[a]	0.805 ± 0.001[a]	135 ± 0.2[a]
	–Zn	0.788 ± 0.035[b]	0.768 ± 0.003[b]	129 ± 0.6[b]

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

Table 2. Gas exchange parameters including net photosynthetic rate (A), transpiration rate (E), stomatal conductance to water vapor (g_s), the ratio of intercellular air space and atmospheric CO_2 molar fractions (C_i/C_a) and instant water use efficiency (WUE, A/E) in red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months with adequate (+Zn) and low (–Zn) Zn supply under control or drought conditions.

Treatments		A (μmol/m^{2}s)	E (mmol/m^{2}s)	g_s (mol/m^{2}s)	C_i/C_a	WUE
Control	+Zn	8.45 ± 0.43^a	2.59 ± 0.98^a	0.46 ± 0.06^a	1.03 ± 0.12^a	3.26 ± 0.99^b
	–Zn	5.09 ± 0.33^b	1.18 ± 0.31^b	0.28 ± 0.17^b	0.83 ± 0.04^a	4.31 ± 1.01^b
Drought	+Zn	4.01 ± 0.21^c	0.95 ± 0.15^b	0.26 ± 0.07^b	1.00 ± 0.01^a	4.22 ± 0.56^b
	–Zn	3.03 ± 0.45^d	0.24 ± 0.21^b	0.23 ± 0.14^b	0.69 ± 0.23^b	12.63 ± 1.22^a

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

tosynthesis rate, the lowest amount of water loss in leaves was observed in Zn-deprived plants under drought conditions. As expected, concomitant with lower transpiration, stomatal conductance declined in Zn-deficient compared with Zn-sufficient leaves. Drought stress also negatively influenced stomatal conductance; however, in Zn-deficient plants water stress did not further affect g_s values. The ratio of C_i/C_a was decreased by low Zn supply only under drought stress. Instant water use efficiency (WUE) was increased by low Zn supply that was significant only in drought-stressed plants. The highest amount of WUE was observed in Zn-deficient plants grown under drought stress (Table 2).

To study the effect of light intensity incident on leaves (Q_{leaf}), gas exchange parameters were determined under different light intensities. The results demonstrated that with increasing Q_{leaf} transpiration rate and particularly net assimilation rate increased continuously in well watered plants irrespective of Zn supply level. In contrast, in drought-stressed plants, increasing Q_{leaf} caused elevated net photosynthesis rate, while it resulted in continuous reduction of transpiration rate, particularly in Zn-deficient plants (data not shown). Calculation of instant water use efficiency ($WUE = A/E$) showed that WUE values increased as the function of increasing Q_{leaf} during gas exchange measurements under all applied treatments; such increase was however more pronounced for –Zn plants under drought stress (Fig. 4).

Table 3. Osmotic potential (ψ_π, MPa) and water potential (ψ_w, MPa) in leaves of red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months with adequate (+Zn) and low (–Zn) Zn supply under control and drought conditions

Treatments		ψ_π	ψ_w
Control	+Zn	–0.87 ± 0.11^a	–0.28 ± 0.01^a
	–Zn	–0.83 ± 0.04^a	–0.29 ± 0.02^a
Drought	+Zn	–1.38 ± 0.09^c	–0.49 ± 0.01^c
	–Zn	–1.10 ± 0.08^b	–0.39 ± 0.02^b

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

Low Zn supply did not affect osmotic potential and water potential in leaves of plants grown under well-watered conditions. In contrast, a significant increase was observed for both water and osmotic potential in drought-stressed plants due to low Zn supply. As expected, water deficit treatment resulted in significantly lower water potential both in Zn-sufficient and Zn-deficient plants. A similar trend was observed for osmotic potential of leaf cell sap (Table 3).

Activities of APX, POD, and CAT were increased not only by low Zn supply but also by water stress; therefore, the greatest amounts of specific activity of these enzymes were detected in low Zn plants suffered from drought stress. In contrast, the activity of SOD was depressed by low Zn but increased under drought stress (Fig. 5).

MDA and H_2O_2 accumulated in response both to Zn deprivation and drought stress; therefore, a great tissue concentration was observed for these metabolites in Zn-deficient plants subjected to water stress. The effect of Zn supply was more pronounced under drought stress compared with well watered conditions. The increase of MDA content due to Zn deprivation was 30% and 70% in well watered and drought-stressed plants, respectively; the corresponding values for H_2O_2 were 68% and 77% (Table 4).

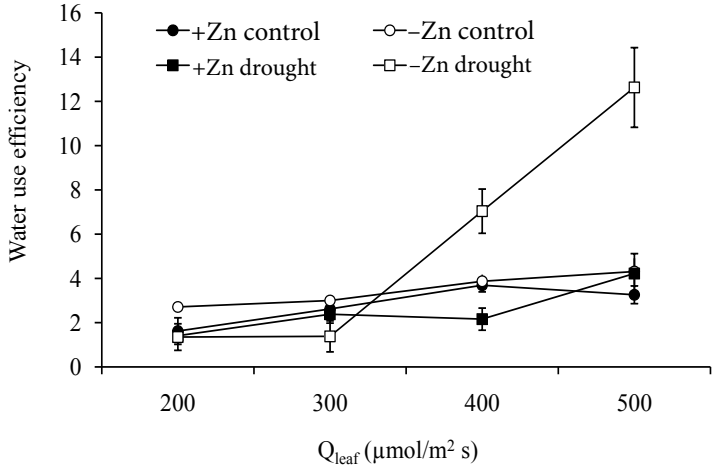

Fig. 4. Instant water use efficiency (A/E) with increasing leaf incident light intensity (Q_{leaf}) in red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply under control and drought conditions. The means refer to 4 repetitions ± SD

DISCUSSION

Red cabbage plants subjected to Zn deficiency conditions showed stunted growth because of shortening of petioles, drastic decrease in leaf surface area (up to 72%) and number of leaves (61% reduction). In Zn-deficient plants, leaf surface area decreased up to 53% when plants were subjected to drought stress, but the number of leaves was not further affected. In contrast to visual symptoms in cereal species that are rather indirect effects of Zn deficiency, 'little leaf' and 'rosetting' symptoms observed in red cabbage similar with other dicotyledons could be considered primary effects of Zn deficiency. Lower leaf surface area could resulted from both impaired cell division and cell expansion due to the effect of Zn in DNA metabolism (Marschner 1995) and loss of membrane integrity resulting in disturbance of ion balance and cell expansion, respectively. Similar explanation was used for the effect of Zn deficiency on the guard cells (Sharma et al. 1995). Lower water availability for cell turgidity and cell wall extension are involved likely in the impaired leaf surface area in Zn-deficient plants subjected to drought stress.

Impairment of growth in Zn-deficient plants was markedly higher when they were subjected to drought stress and in turn, the effect of drought stress on the inhibition of dry matter production was greater in Zn-deficient compared with Zn-sufficient plants. Drought stress-mediated reductions of shoot and root dry weight were 72% and 50% for Zn-deficient, and 65% and 46% for Zn-sufficient plants, respectively. Possible reasons of the intensifying effect of drought stress will be discussed below.

Plant total Zn content, i.e. Zn uptake per plant, declined dramatically not only at low Zn supply but also under drought conditions. Zinc transport to root surfaces, which is mainly via diffusion, is expected to decrease under drought conditions. It was reported that Zn deficiency is prone to occur in arid and semi-arid regions where soils, particularly top soil, are usually deficient in water (Cakmak et al. 1996). In addition, under low Zn and drought stress only a small part of Zn taken up by plants was transported into leaves. Significantly lower stomatal opening and transpiration probably caused reduction of transport of minerals in general and of Zn in particular into shoot. Greater reduction of Zn con-

Table 4. Concentration of MDA (nmol/g FW), hydrogen peroxide (µmol/g FW) and proline (µmol/g FW) in leaves of red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months with adequate (+Zn) and low (–Zn) Zn supply under control and drought conditions

Treatments		MDA	H_2O_2	Proline
Control	+Zn	4.82 ± 0.70^{d}	7.65 ± 0.50^{c}	2.63 ± 0.60^{c}
	–Zn	6.28 ± 1.10^{c}	12.84 ± 0.75^{b}	5.89 ± 0.70^{b}
Drought	+Zn	11.91 ± 3.50^{b}	12.24 ± 0.72^{b}	6.04 ± 0.84^{b}
	–Zn	20.23 ± 6.63^{a}	21.72 ± 0.72^{a}	14.07 ± 2.12^{a}

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

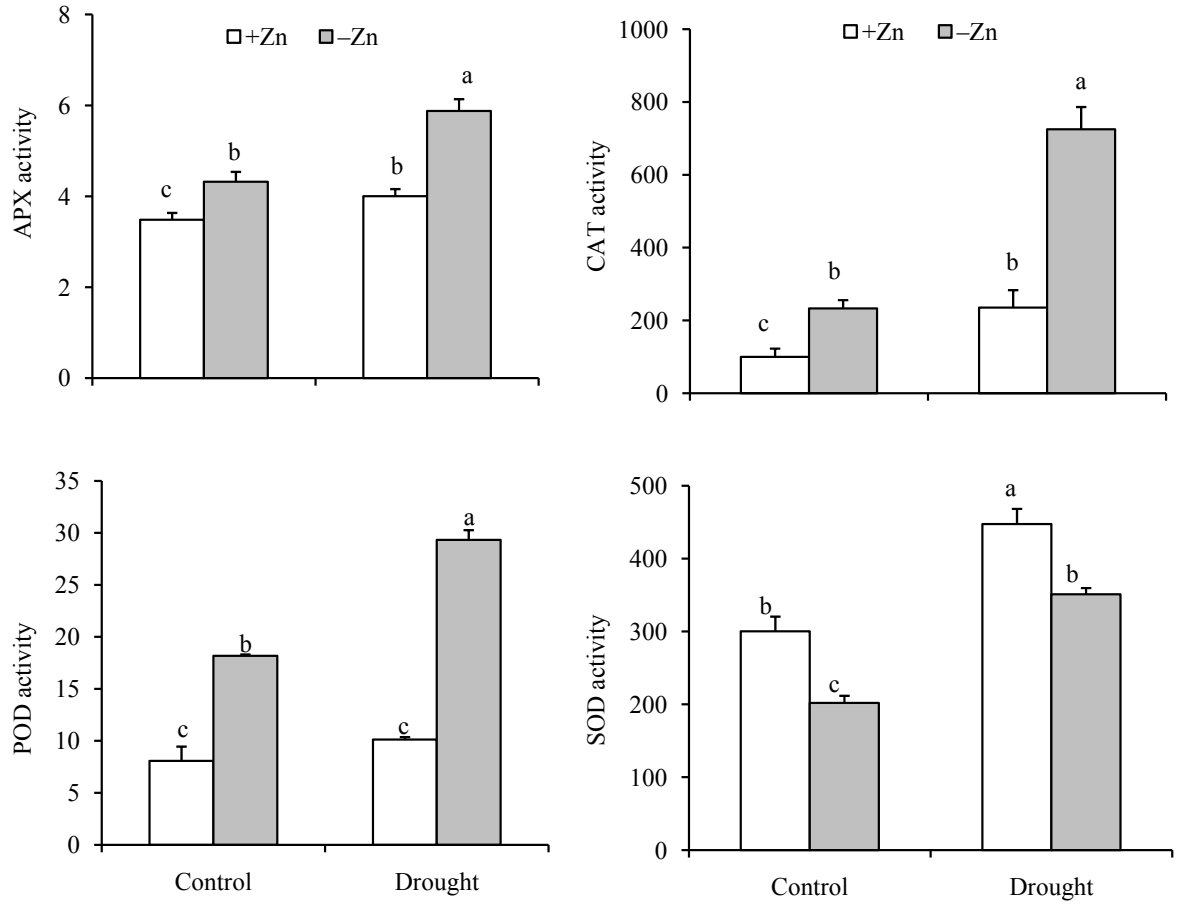

Fig. 5. Specific activity of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in the shoot and roots of red cabbage (*Brassica oleraceae* L. var. *capitata* f. *rubra*) plants grown for two months in nutrient solution with adequate (+Zn) and low (–Zn) Zn supply under control and drought conditions. The means refer to 4 repetitions ± SD. Bars indicated by the same letter are not significantly different ($P < 0.05$)

tent in leaves is likely one of the reasons for higher susceptibility of shoot growth to low Zn supply and drought conditions compared with roots.

The initial Chl fluorescence yield (F_0), that reflects the minimal fluorescence yield when all Q_A are in oxidized state, was reduced both by low Zn supply and drought stress. In addition, Zn-deficient leaves had significantly smaller maximal fluorescence yield (F_m) value compared to the control, demonstrating likely a diminished pool of plastoquinone 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 photosynthesis processes conserved their normal activities in Zn-deprived leaves. For nutrients without direct involvement in the electron transport or chlorophyll synthesis such as Zn, a close linkage between nutritional status of leaves and spectral characteristics seems unlikely (Adams et al. 2000). However, in leaves that experienced the dual effect of low Zn and drought stress, reduction of F_v/F_m demonstrated a serious damage to photosynthetic apparatus. In contrast, Zn-sufficient plants responded to drought stress by increasing $\Phi_{PS\,II}$ and ETR suggesting that drought stress exerted no damaging effect on photochemical events when leaves are supplied with adequate Zn. Drought did not influence other fluorescence parameters in Zn-sufficient plants negatively (with the exception of F_0), which further supports this hypothesis.

Reduction of photochemical quenching by low Zn treatment could be related to photoinhibition rather than to a direct damage to PS II (Baker, Bowyer 1994). One of the causes of photoinhibition in Zn-deficient plants could be lower leaf Chl content which has a crucial role for the susceptibility to photoinhibition (Pätsikkä et al. 2002). Non-photochemical fluorescence quenching is one of the mechanisms to prevent or alleviate damage to the photosynthetic apparatus. In our experiment,

Zn deficiency conditions likely cause an excess of reducing power (NADP + H^+) because of either a decrease in the CO_2 available at carboxylation sites i.e. stomatal closure, or sink limitation i.e. lower demand because of impaired growth. 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 ROS, which further injures PS II components, is very high. Although in this experiment evidence showed no damage to photosynthetic apparatus (with the exception of leaves subjected to drought stress and low Zn) so far, it is expected that with more severe Zn deficiency or longer growth period under deficiency conditions, serious damage would occur to photosystems in Zn-deficient leaves.

Both low Zn supply and drought stress caused similar effects on the gas exchange parameters. Lower net CO_2 uptake and transpiration were well correlated with impaired stomatal conductance only in well-watered plants. Stomatal limitation due to drought stress was greater for Zn-sufficient plants (43% g_s reduction) compared with Zn-deficient ones (11% g_s reduction, non-significant). It suggested that impairment of stomatal conductance was the most important cause for by 53% lower net CO_2 uptake in Zn-sufficient plants subjected to drought stress. In contrast, a 40% reduction of net CO_2 uptake in Zn-deficient plants due to drought stress that was not associated with declined stomatal conductance resulted mainly from non-stomatal limitations i.e. inhibited photochemical reactions. Reduction of C_i/C_a and a marked increase in *WUE* showed that plants under low Zn supply are more conservative in relation to water economy than sufficient plants when grown under drought stress. However, concomitant with lower CO_2 uptake per surface area of leaf (A), the remarkable reduction of total plant leaf area may affect greatly whole plant photosynthesis, contributing to the low biomass production under dual effect of low Zn and drought stress.

Lower water loss in Zn-deficient plants due to greater stomatal limitation led to greater water and osmotic potential compared with Zn-sufficient plants when subjected to drought stress. Reduction of water and osmotic potential due to drought stress was 75% and 59% for Zn-sufficient and only 34% and 33% for Zn-deficient plants, respectively. In addition, a significant increase in proline accumulation was observed in Zn-deficient plants subjected to drought stress. Proline as a compatible osmolyte serves as a protectant for enzymes and cellular structures (Hasegawa et al. 2000). Results imply that Zn-deficient plants subjected to drought stress seemed to be more protected against drought stress than Zn-sufficient plants because of lower water loss, greater water and osmotic potential and higher leaf proline content. However, greater susceptibility to low Zn supply in plants suffering from drought stress indicated that these factors are not critical for determination of plant growth response.

Activity of all antioxidant enzymes, with the exception of SOD, enhanced under low Zn supply. 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 et al. 1997). Drought conditions enhanced the activity of studied enzymes including SOD. Yet, a marked increase in MDA and H_2O_2 content, a significant increase in the activity of H_2O_2 scavenging enzymes and SOD as well as accumulation of proline as a potent scavenger of hydroxyl radicals implies a serious imbalance between ROS production and destruction (Alia, Mohanty 1997). It indicated insufficient ability of antioxidant enzymes for scavenging ROS particularly under combinative effect of low Zn and drought conditions.

Results demonstrated that a strong decline in leaf Zn content, damage of photosynthesis apparatus, remarkable reduction of whole plant photosynthesis and imbalance between ROS production and scavenging are important factors contributing to growth reduction of red cabbage plants under combinative effects of Zn deficiency and drought stress. In contrast, higher *WUE* and greater water and osmotic potential of leaves did not seem to be critical for determination of plant growth response under these conditions.

References

Adams M.L., Norvell W.A., Philpot W.D., Peverly J.H., 2000. Spectral detection of micronutrient deficiency in 'Bragg' soybean. Agronomy Journal, *92*: 261–268.

Alia P.S.P., Mohanty P., 1997. Involvement of proline in protecting thylakoid membrane against free radical-induced photodamage. Journal of Photochemistry & Photobiology B: Biology, *38*: 253–257.

Apel K., Hirt H., 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology, *55*: 373–399.

Bağci S.A., Ekiz H., Yilmaz A., Cakmak I., 2007. Effect of Zn deficiency and drought on grain yield of field grown

wheat cultivars in Central Anatolia. Journal of Agronomy and Crop Science, *193*: 198–206.

Baker N.R., Bowyer J.R., 1994. Photoinhibition of Photosynthesis. From Molecular Mechanisms to the Field. Oxford, BIOS Scientific Publishers.

Bruce W.B., Edmeades G.O., Baker T.C., 2002. Molecular and physiological approaches to maize improvement for drought tolerance. Journal of Experimental Botany, *53*: 13–25.

Cakmak I., Yilmaz A., Kalayaci M., Ekiz H., Torun B., Erenoglu B., Braun H.J., 1996. Zn deficiency as a critical problem in wheat production in central Anatolia. Plant and Soil, *180*: 165–172.

Cakmak I., Öztürk L., Eker S., Torun B., Kalfa H.I., Yilmaz A., 1997. Concentration of zinc and activity of copper/zinc superoxide dismutase in leaves of rye and wheat cultivars differing in sensitivity to zinc deficiency. Journal of Plant Physiology, *151*: 91–95.

Cakmak I., 2000. Possible roles of Zinc in protecting plant cells from damage by reactive oxygen species. New Phytologist, *146*: 185–205.

Chen W., Yang X., He Z., Feng Y., Hu F., 2007. Differential changes in photosynthetic capacity, 77K chlorophyll fluorescence and chloroplast ultrastructure between Zn-efficienct and Zn-inefficient rice genotypes (*Oryza sativa* L.) under low Zn stress. Physiologia Plantarum, *132*: 89–101.

Genty B., Briantais M.J., Baker N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta, 990: 87–92.

Hajiboland R., Beiramzadeh N., 2008. Growth, gas exchange and function of antioxidant defense system in two contrasting rice genotypes under Zn and Fe deficiency and hypoxia. Acta Biologica Szegediensis, *52*: 283–294.

Hajiboland R., Hasani, B.D., 2007. Responses of antioxidant defense capacity and photosynthesis of bean (*Phaseolus vulgaris* L.) plants to copper and manganese toxicity under different light intensities. Acta Biologica Szegediensis, *51*: 93–106.

Hasegawa P.M., Bressan R.A., Zhu J.K., Bohnert H.J., 2000. Plant cellular and molecular response to high salinity. Annual Review of Plant Physiology, Plant Molecular Biology, *51*: 463–499.

Johnson C.M., Stout P.R., Broyer T.C., Carlton A.B., 1957. Comparative chloride requirements of different plant species. Plant and Soil, *8*: 337–353.

Kaur C., Kapoor H.C., 2001. Antioxidants in fruits and vegetables-the millenium's health. Internal Journal of Food Science & Technology, *36*: 703–725.

Krall J.P., Edwards G.E., 1992. Relationship between photosystem II activity and CO_2 fixation in leaves. Physiologia Plantarum, *86*: 180–187.

Lichtenthaller H.K., Wellburn A.R., 1985. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biological Society Transactions, *11*: 591–592.

Marschner H., 1995. Mineral Nutrition of Higher Plants. 2nd Ed. London, Academic Press Inc.

Maxwell K., Johnson G.N., 2000. Chlorophyll fluorescence–A practical guide. Journal of Experimental Botany, *51*: 659–668.

Magné C., Saladin G., Clément C., 2006. Transient effect of the herbicide flazasulfuron on carbohydrate physiology in *Vitis vinifera* L. Chemosphere, *62*: 650–657.

Oxborough K., 2004. Imaging of chlorophyll *a* fluorescence: theoretical and practical aspects of an emerging technique for the monitoring of photosynthetic performance. Journal of Experimental Botany, *55*: 1195–1205.

Ouzounidou G., Ilias I., Kabataidid M., Chatzimichail A., 2003. Comparative study of nutrient deficiencies on growth and photochemistry of tobacco. Journal of Plant Nutrition, *26*: 1605–1616.

Parker D.R., Norvell W.A., Chaney R.L., 1995. GEOCHEM-PC: A chemical speciation program for IBM and compatible computers. In: Leoppert R.H. et al. (eds.), Soil Chemical Equilibrium and Reaction Models. SSSA Special Publication No. XX. Madison, WI, Soil Science Society of America.

Pätsikkä E., Kairavuo M., Šeršen F., Aro E-M., Tyystjärvi E., 2002. Excess copper predisposes photosystem II to photoinhibition *in vivo* by outcompeting iron and causing decrease in leaf chlorophyll. Plant Physiology, *129*: 1359–1367.

Sharma, P.N., Tripathi A., Bisht S.S., 1995. Zinc requirement for stomatal opening in cauliflower. Plant Physiology, *107*: 751–756.

Tennant D., 1975. A test of modified line intersect method of estimating root length. Journal of Ecology, *63*: 995–1001.

Wang H., Liu R.L., Jin J.Y., 2009. Effects of zinc and soil moisture on photosynthetic rate and chlorophyll fluorescence parameters of maize. Biologia Plantarum, *53*: 191–194.

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