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Photosynthetic rate, chlorophyll fluorescence parameters, and lipid peroxidation of maize leaves as affected by zinc deficiency

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Abstract

Pot trial in greenhouse was conducted using cumulic cinnamon soil from North China to study the effects of zinc deficiency on CO_2 exchange, chlorophyll fluorescence, the intensity of lipid peroxidation, and the activity of superoxide dismutase (SOD) in leaves of maize seedlings. Zn deficiency resulted in a reduction of net photosynthetic rate and stomatal conductance to H_2O. The maximum quantum efficiency of photosystem 2 (PS2) and the PS2 activity were depressed, while the pool size of the plastoquinone molecules was not affected by Zn deficiency. The content of super oxygen anion radical ($O_2^{\cdot-}$) and the intensity of lipid peroxidation as assessed by malonyldialdehyde content in Zn-deficient leaves were higher than those in Zn-sufficient leaves. The activity of SOD increased with Zn application. The adverse influence of Zn-deficiency on the light stage of photosynthesis is probably one of possible reasons for the limitation of photosynthetic capacity in maize leaves.

Additional key words: photosystem 2; plastoquinone; stomatal conductance to H_2O; superoxide dismutase; superoxygen anion radical; *Zea*.

Introduction

Zinc is essential for plant growth and function. Zn-deficiency is one of the most important micronutrient stresses limiting crop growth and productivity worldwide (Takkar and Walker 1993, Alloway 2001). Zn deficiency depresses plant leaf's photosynthetic capacity. In cauliflower, reduction in photosynthesis induced by Zn deficiency is associated with a decrease in intercellular CO_2 concentration and stomatal conductance (Sharma *et al.* 1994). Sharma *et al.* (1995) reported a significant role of Zn in the regulation of the stomatal aperture, which is accounted for possible role of Zn in maintaining a high K content in guard cells. A decrease in carbonic anhydrase activity due to Zn deficiency may also contribute to the reduced net photosynthetic rate, P_N (Ohki 1976, Rengel 1995, Cakmak and Engels 1999, Hacisalihoglu *et al.* 2003). Fischer *et al.* (1997) showed a higher P_N in Zn-deficiency resistant wheat cultivars than in a sensitive cultivar was related to higher carbonic anhydrase activity, because irrespective of Zn supply the resistant cultivar had an inherently higher carbonic anhydrase activity than the sensitive cultivar (Rengel 1995). Additionally, the accumulation of saccharides in leaves may be related to decreases in photosynthetic CO_2 fixation under low Zn (Marschner 1995, Cakmak 2000). The accumulation of saccharides can be observed in Zn deficient plants, especially in phloem sap source leaves, possibly resulting from either impaired phloem export of saccharides or decrease of sink demand (Cakmak 2000).

Recently, chlorophyll (Chl) fluorescence measurements have been used to estimate, rapidly and non-invasively, the operating quantum efficiency of electron transport through photosystem 2 (PS2) in leaves of plants. This PS2 operating efficiency is related to CO_2 assimilation (Maxwell and Johnson 2000, Sayed 2003, Baker and Rosenqvist 2004). The deficiencies of some micronutrient metals such as Mn, Fe, and Cu cause changes of Chl fluorescence reactions in leaves (Adams *et al.* 2000, Balakrishnan *et al.* 2000, Henriques 2003). Mn and Fe are involved directly in electron transport reactions and are essential for the synthesis of Chl (Spiller *et al.* 1982, Pushnik *et al.* 1989). Cu is also directly involved in electron transport reactions as an essential component of plastocyanin (Droppa and Horváth 1990). However, there is little information on whether Zn deprivation may

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diminish the leaf photosynthetic capacity by depressing PS2 photochemical ability or by affecting the light stage of photosynthesis.

Maize is one of crops sensitive to Zn deficiency, and severe Zn deficient symptoms such as stunted and chlorotic plants are often observed in early grown maize plants in the field (Alloway 2001). In this study, maize was selected as tested plant to examine P_N and Chl fluorescence characteristics of normal and Zn-deficient maize leaves of early maize seedlings grown in pot experiment using Zn-deficient soil. The extent of lipid peroxidation, the level of super oxygen anion radical ($O_2^{\cdot-}$), and the activity of SOD were determined in control and Zn-stressed leaves of maize.

Materials and methods

Tested soil: Cumulic cinnamon soil with heavy texture was sampled from Shanxi Province in Northern China. The soil pH (1 : 5, soil : H_2O) was 7.9, the organic matter C 8.0 g kg^{-1}, $CaCO_3$ 15.3 g kg^{-1}, the concentration of soil DTPA-Zn prior to cropping was 1.3 mg kg^{-1}. Soil saturated water capacity was 36 % (m/m) of soil moisture content. 4.0 kg of air-dried soils passed through a 2-mm nylon sieve were weighed and placed into polyethylene plastic pots with 14.6-cm diameter and 20-cm height.

Plants: Maize (cv. Zhongdan 9409) seeds of similar size were surface-sterilised by soaking in 10 % H_2O_2 for 30 min, washed thoroughly in de-ionized water, and germinated on the filter paper at 25 °C for 48 h. Initially, seven uniformly germinated seeds were sown in each pot and later thinned to five plants per pot. Plants were grown in Beijing greenhouse from 15 August to 15 October of 2001. Temperature within the greenhouse was 33±3 ºC during the day and 17±3 ºC during the night. Plants were grown under natural day length. After the three leaf stage, 0.16 g kg^{-1} N, 0.12 g kg^{-1} P_2O_5, and 0.08 g kg^{-1} K_2O as solution prepared with urea and KH_2PO_4 were added to soil.

Zn treatments of 0 and 5.0 mg Zn per kg soil were applied as $ZnSO_4 \times 7\ H_2O$ solution, which were represented by symbols Zn_0 and Zn_5, respectively.

Harvest and chemical analysis: Plants were harvested and divided into shoots and roots. Soil was washed out of the roots. These plant samples were rinsed using distilled water, killed at 105 ºC for 30 min, dried at 65~70 ºC for 2 d, and weighed. Dry plant materials were digested in HNO_3-$HClO_4$ and the extract was analyzed for Zn using atomic absorption spectrophotometer. The experiment was set up in a completely randomized factorial design (2 Zn levels×4 replicates). Three replicates from each treatment were used for analysis.

Chl was extracted with 80 % acetone in the dark for 48 h at 25 ºC until plants were blanched. The equations of Porra *et al*. (1984) were used for quantification.

Gas exchange measurements: P_N and transpiration rate (E) of attached leaves were measured with an open flow-through portable system (*LCA-4, ADC Bio Scientific*) at ambient CO_2 pressure. The first fully expanded leaf was measured after the Zn deficiency symptoms occurred in plants. All measurements were carried out between 09:00 and 11:00. During the measurements the air relative humidity was about 70 %, the leaf temperature ranged between 25–28 ºC, and the ambient CO_2 concentration was 320~380 µmol mol^{-1}.

Chl fluorescence parameters were recorded in parallel to gas exchange measurements in the same leaf, using a portable fluorometer (*PEA*, *Hansatech*, Kings Lynn, Norfolk, England). It is a compact, continuous-excitation type Chl fluorescence analyzer. Leaves were acclimated to dark for 20 min before measurements were taken. The time of measuring was 5 s, and irradiance was set at 75 % of maximum (>3 000 µmol m^{-2} s^{-1}). Initial (F_0), maximum (F_m), variable ($F_v = F_m - F_0$), F_v/F_0, and F_v/F_m were recorded. F_v/F_m was used to indicate potential maximal quantum yield of PS2, F_v/F_0 was used to assess PS2 activity. The area over the fluorescence curve between F_0 and F_m was also recorded and the relative pool size of plastoquinone (PQ) was estimated from the ratio of this area to the value of $F_m - F_0$.

Determination of $O_2^{\cdot-}$ level and superoxide dismutase (SOD) activity: In the beginning of Zn-deficiency symptom, the fully expanded young leaves were collected near midday, immediately frozen in liquid nitrogen, and stored at –80 °C until analysis. 1~2 g of leaves were ground with a pestle in an ice-cold mortar with 10 cm^3 of 0.05 M sodium phosphate buffer (pH 7.8). The homogenates were filtered through four layers of cheese cloth and then centrifuged at 4 ºC for 20 min at 15 000×*g*. The supernatants were used for the $O_2^{\cdot-}$ determination and the assays of SOD enzyme activity at 25 ºC.

The above extract was also used to determine the content of $O_2^{\cdot-}$ based on hydroxylamine oxidation reaction $NH_2OH + 2\ O_2^{\cdot-} + H_2O \rightarrow NO_2^{\cdot-} + 2\ H_2O_2$. The production of $NO_2^{\cdot-}$ was determined by colorimetry (Wang and Luo 1990).

The SOD activity was measured spectrophotometrically as described by Beyer and Fridovich (1987) according to its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). One unit of SOD was defined as the amount required to inhibit the photoreduction of NBT by 50 %.

Membrane lipid peroxidation in the leaves was

estimated using malonyldialdehyde (MDA), which is a decomposition product from the peroxidation of poly-unsaturated fatty acids. Leaves were ground in a mortar with liquid nitrogen and extracted with 10 % trichloro-acetic acid (TCA). After centrifuging at 4 000×*g* for 10 min at 4 ºC, supernatants were pooled and an aliquot of appropriately diluted sample was added to a test tube to react with 0.6 % (m/v) thiobarbituric acid (TBA) solution comprising 10 % TCA. Samples were heated at 95 ºC for 15 min and after cooling, absorbances were read at 450, 532, and 600 nm. Malondialdehyde equivalents [μM] were calculated as 6.45 $(A_{532} - A_{600}) - 0.56\ A_{450}$. MDA content in leaves was calculated per fresh leaf mass (Zhu *et al.* 1990).

Results

Plant growth: After the five to six leaf stage, Zn-deficient symptoms appeared in maize plants. Chlorosis of young leaves followed by white necrotic spots was observed on the leaf blades. Plant height and internodal length of Zn-deficient plants were affected substantially. Zn application increased significantly the dry matter of shoots, but did not affect root dry matter yet. Root to shoot ratio increased under Zn-deficiency (Table 1).

Table 1. Dry matter [g per pot] of maize plants as affected by Zn. Means ± SD. $^{*,\ **}$, and ns represent $p<0.05$, $p<0.01$, and not significant, respectively.

Treatment	Shoots	Roots	Total plants	Root/shoot ratio
Zn_0	3.772±0.635	1.874±0.465	5.646±1.046	0.495±0.071
Zn_5	6.696±0.954	1.860±0.273	8.556±1.225	0.278±0.007
Significance	*	ns	*	**

Zn contents in roots and shoots increased significantly by Zn addition (Table 2), while the Zn content in shoots without Zn application was below critical level (20 mg kg^{-1}) of Zn deficiency (Alloway 2001).

The Zn content in roots and shoots increased with Zn application, moreover, Zn accumulation in shoots due to Zn application was relatively higher than in roots. With Zn application, the % Zn uptake by roots and shoots in total plant Zn uptake was 67 and 33 %, respectively, while such percentage was 53 and 47 % under Zn-deficiency. Thus Zn application might stimulate Zn transport from roots to shoots.

Table 2. Zn contents as affected by Zn.

Treatment	Zn [mg kg^{-1}]		Zn [μg per pot]		
	Shoots	Roots	Shoots	Roots	Total plants
Zn_0	16.10±1.24	29.62±1.59	61.21±14.12	55.10±11.30	116.31±25.13
Zn_5	27.24±2.64	48.01±3.25	182.95±34.23	89.52±16.75	272.47±48.16
Significance	**	**	**	*	**

***P*$_N$, *E*, and stomatal conductance (*g*$_s$)**: Zn-deficiency strongly reduced the photosynthetic performance in maize leaves: P_N, E, and g_s were decreased by 80, 62, and 69 %, respectively, in comparison to Zn_5. The water use efficiency, as assessed by P_N/E, was enhanced markedly by Zn application (Table 3).

Table 3. Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) as affected by Zn.

Treatment	P_N [μmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	$P_N/E\times10^{-3}$	g_s [mmol m^{-2} s^{-1}]
Zn_0	1.74±0.65	0.50±0.07	3.45±0.90	13.77±2.09
Zn_5	10.16±0.60	1.55±0.07	6.57±0.16	52.92±4.09
Significance	**	**	**	**

Leaf Chl *a* and *b* contents were decreased by Zn-deficiency (Table 4). Zn-deficiency resulted in greater reduction of Chl *b* than Chl *a* and so Chl *a*:*b* increased in Zn_0 leaves.

Table 4. Effects of Zn on chlorophyll (Chl) contents [g kg^{-1}(f.m.)] in leaves of maize plants.

Treatment	Chl *a*	Chl *b*	Chl (*a*+*b*)	Chl *a*/*b*
Zn_0	1.54±0.01	0.59±0.01	2.14±0.01	2.61±0.05
Zn_5	1.58±0.01	0.67±0.01	2.25±0.02	2.37±0.03
Significance	*	**	**	*

Chl fluorescence parameters of leaves are presented in Table 5. F_v and F_m of Zn_0 leaves were lower than those of Zn_5 leaves, while F_0 and T_m were not altered with Zn treatments. F_v/F_m declined significantly in the Zn_0 leaves compared with Zn_5, indicating that the potential maximal quantum yield of PS2 was inhibited by Zn-deficiency. F_v/F_0 under Zn-deficiency was also below the normal Zn supply, implying PS2 activity was reduced by Zn-deficiency. The relative pool size of PQ molecules associated with PS2, which was calculated as the ratio of the area above the fluorescence induction curve to the value of $F_m - F_0$, was not affected by Zn-deficiency.

Table 5. Chlorophyll (Chl) fluorescence induction parameters as affected by Zn: F_0: minimal fluorescence, F_v: variable fluorescence, F_m: maximal fluorescence, T_m: the time taken for the Chl fluorescence rise from its initial to maximum level. Relative PQ pool size was estimated from the ratio of the area above the fluorescence induction curve to the value of $F_m - F_0$.

Treatment	F_0	F_v	F_m	F_v/F_m	F_v/F_0	T_m [ms]	Relative PQ pool
Zn_0	601.8±33.0	1951.0±119.1	2552.8±147.6	0.764±0.006	3.243±0.114	268.8±29.2	23.9±3.1
Zn_5	629.4±25.2	2633.6±87.2	3263.0±87.9	0.806±0.009	4.190±0.226	284.4±15.2	24.5±3.7
Significance	ns	**	**	**	**	ns	ns

MDA concentration, the $O_2^{\cdot-}$ production, and the activity of SOD: Zn-deficiency caused the increase in $O_2^{\cdot-}$ production and higher contents of MDA in leaves. The SOD activity showed a decrease in Zn_0 leaves, indicating that lowered capacity to scavenge the free radicals may be related to the over-accumulation of reactive oxygen species (ROS), which enhanced the rate of membrane peroxidation, *i.e.* MDA content in Zn_0 leaves (Table 6).

Table 6. Malonyldialdehyde (MDA) content, $O_2^{\cdot-}$ production, and the activity of superoxide dismutase (SOD) as affected by Zn.

Treatment	MDA [μmol kg^{-1}(f.m.)]	$O_2^{\cdot-}$ production [mol s^{-1} kg^{-1}(protein)]	SOD [U mg^{-1}(protein)]
Zn_0	5.54±0.24	10.17±0.33	23.82±3.46
Zn_5	4.63±0.10	8.00±0.67	36.28±0.39
Significance	**	**	**

Discussion

The reasons for Zn-deficiency depressed plant leaf photosynthetic capacity may be associated to the decrease in intercellular CO_2 concentration, stomatal conductance (Sharma *et al.* 1994, 1995), and the decrease in carbonic anhydrase activity (Ohki 1976, Rengel 1995, Fischer *et al.* 1997, Cakmak and Engels 1999, Hacisalihoglu *et al.* 2003). In addition, the accumulation of saccharides in leaves may be an important factor for the inhibition of photosynthesis under Zn-deficiency (Marschner 1995, Cakmak 2000). We found that P_N of Zn_0 leaves was significantly lower than that of Zn_5 leaves. The decrease of g_s was also observed in Zn-deficient leaves.

Chl fluorescence kinetics showed that the maximum quantum efficiency of PS2 and the activity of PS2 estimated by F_v/F_m and F_v/F_0 were reduced by Zn-deficiency. Not only the dark stage of photosynthesis, but also the light stage of photosynthesis was inhibited by Zn-deficiency.

Sharma *et al.* (2004) proposed that oxidative stress is an early sign of plants when they are subjected to Zn-deficiency because the induction of anti-oxidative responses to Zn-deficiency occurred rapidly and before symptoms of severe Zn-deficiency. The mechanisms by which Zn-deficiency damages plants results from appearance of ROS (for review see Cakmak 2000): (*1*) Under Zn-deficiency, the activity of membrane-bound NADPH oxidase producing ROS increases (Cakmak and Marschner 1988) and the activity of SOD decreases (Yu *et al.* 1998). (*2*) The higher iron concentration in Zn deficient plants (Cakmak *et al.* 1996) potentates the production of free

radicals through Fe-catalysed Haber-Weiss reaction (Price and Hendry 1991). (*3*) Plant photo-oxidation can be enhanced by Zn-deficiency and so those leaves with Zn-deficiency are highly light-sensitive (Marschner and Cakmak 1989). Our study also demonstrated a higher production of $O_2^{\cdot -}$ and higher contents of MDA in Zn-deficient leaves. Zn application significantly enhanced SOD activities and reduced the levels of $O_2^{\cdot -}$. The enhanced ROS formation is harmful for chloroplast and causes the destruction of many chloroplast constituents (Bukhov 2004). Jin and Tao (2000) reported the decrease in F_v/F_m is accompanied by an enhanced production of superoxide. Henriques (2001) showed that in sugar beet leaves, increasing Zn shortage induces extensive disorganization of chloroplast thylakoids, followed by their degradation as well as that of stroma components. They also proposed that the reduction in the blade's photosynthetically active area, together with a decline in the photochemical capability of the chloroplasts from the remaining leaf area, are the primary causes for the observed reduction in P_N. It suggested that the higher contents of ROS under Zn-deficiency may lead to the damage of the light-harvesting complex and the reaction centre PS2, and inhibit the PS2 photochemistry ability and electron transport. However, this possibility should be further confirmed.

In conclusion, Zn-deficiency inhibited the maximum quantum efficiency of PS2 and the activity of PS2, while it did not affect the relative pool size of the PQ molecules. It suggested that the adverse effect of Zn-deficiency on the light stage is one of possible reasons for the limitation of photosynthetic capacity in maize leaves.

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