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BRIEF COMMUNICATION

Effects of zinc and soil moisture on photosynthetic rate and chlorophyll fluorescence parameters of maize

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Abstract

Effects of zinc [0 and 5.0 mg Zn kg^{-1} (soil)] on photosynthetic rate (P_N), and chlorophyll fluorescence in leaves of maize (*Zea mays* L.) cv. Zhongdan 9409 seedlings grown under different soil moisture regimes (40 - 45 % and 70 - 75 % of soil saturated water content) were studied. Zn application did not enhance maize plant adaptation to drought stress. The relative water content and the water potential of leaves were not affected by Zn treatment. Moreover, The P_N of drought-stressed plants was not improved by Zn supply. The increases of plant biomass, stomatal conductance and quantum yield of photosystem 2 due to Zn addition were notable in well-watered plants.

Additional key words: drought, photosystem 2, stomatal conductance, *Zea mays*, zinc deficiency.

In some maize-growing areas plants suffers from drought stress at different life stages. The decline of net photosynthetic rate (P_N) induced by water stress was often observed. This decline may be related to a reduction in light interception due to lower leaf area, to reduction in carbon fixation per unit leaf area or to damage of the photosynthetic apparatus (Lal and Edwards 1996, Saccardy *et al*. 1996, Foyer *et al*. 1998, Castrillo *et al*. 2001, Bruce *et al*. 2002). The soil in dry regions is often poor in plant-available zinc (Zn) associated with high calcium carbonate content and alkaline pH (Liu 1996). Zn deficiency symptoms such as stunted stems and chlorotic leaves were often observed in maize plants grown in the field (Liu *et al*. 1993, Liu 1996). In cauliflower, a reduction in photosynthesis induced by Zn deficiency was associated with a decrease in stomatal conductance (g_s) and intercellular CO_2 concentration (Sharma *et al*. 1994). A decrease of carbonic anhydrase activity due to Zn deficiency also contributed to the reduced 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 that was related to higher carbonic anhydrase activity. In cabbage, Zn deficiency lowered osmotic potential and increased water saturation deficit (Sharma *et al*. 1984, 1994). The transpiration rate (E) of pecan plants declined under Zn deficiency (Hu and Sparks 1991). Grewal and Williams (2000) showed that the ability of alfalfa plants to cope with water stress during early vegetative stage could be enhanced with adequate Zn supply. However, Khan *et al*. (2003) reported that applying Zn increased chickpea grain yields when the plants were well-watered, but not under water stress, except for the Zn-efficient and drought-resistant genotype ICC-4958. Therefore, the aim of this research was to examine the responses of growth, water status, and photosynthesis of maize to Zn addition under well-watered and drought conditions.

Pots were filled with cumulic cinnamon soil sampled from Shaanxi Province in Northwest China. The soil pH was 7.9, organic carbon 8.0 g kg^{-1}, $CaCO_3$ 15.3 g kg^{-1}. The concentration of soil DTPA-Zn prior to cropping was

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Abbreviations: Chl - chlorophyll; DAP - days after planting; E - transpiration rate; F_0 - initial fluorescence; F_v - variable fluorescence; F_m - maximal fluorescence; g_s - stomatal conductance; P_N - photosynthetic rate; PS - photosystem; RWC - relative water content; T_m - the time taken for the chlorophyll fluorescence rise from its initial to maximum level.

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1.3 mg kg^{-1} (below the critical level of available soil Zn). Soil saturated water capacity was 36 % (m/m). Seeds of maize (*Zea mays* L.) cv. Zhongdan 9409 were surface-sterilised by soaking in 10 % H_2O_2 for 30 min, washed thoroughly in de-ionised water, and germinated on the filter paper at 25 °C for 48 h. Plants were grown under natural day length in greenhouse with day/night temperature of about 30/20 °C. After the three-leaf stage, 0.16 g kg^{-1} N, 0.05 g kg^{-1} P, 0.07 g kg^{-1} K were added.

Two Zn treatments were set: no applied Zn (Zn_0) and 5.0 mg Zn kg^{-1}(soil) applied as $ZnSO_4 \cdot 7H_2O$ solution before sowing (Zn_5). Two soil moisture treatments 40 - 45 % (water-stressed condition) and 70 - 75 % (well-watered condition) of soil moisture capacity were imposed after the four-leaf stage till harvest. De-ionized water was used for irrigation. Soil moisture was controlled by gravimetric method every day and water was added when necessary. To minimize the effects of differences in plant size induced by the Zn treatments on the development of water stress, time domain reflectometry (*TRASE*, *Soil Moisture Equipment Corp*., Santa Barbara, USA) with 15 cm length wave-guides was used as other method to measure soil moisture. The soil density in pots was estimated as 1.70 g cm^{-3}.

The leaf water potential was measured using psychrometers. The youngest and fully expanded leaves were sampled at about 10:00 a.m. from the plants at 40 d after planting (DAP). Relative water content (RWC) was calculated as: (FM - DM)/(TM - DM) × 100, where FM was the fresh mass, TM was the mass after rehydrating samples for 24 h by soaking the leaves in water, and DM was the dry mass obtained after oven-drying samples at 75 °C for 48 h. P_N, E and g_s of attached leaves were measured with a portable system *LCA-4*, (*ADC Bio Scientific,* Hoddesdon, UK). The first fully expanded leaf was selected to measure at 40 DAP when Zn deficiency and water 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 25 - 28 °C, and the ambient CO_2 concentration 320 - 380 µmol mol^{-1}. A portable fluorometer (*PEA*, *Hansatech*, Kings Lynn, Norfolk, England) was used to measure chlorophyll (Chl) fluorescence parameters in parallel to gas exchange measurements in the same leaf. Leaves were acclimated to dark for 20 min before measurements were taken. The measuring time was 5 s, and the irradiance was set at 75 % of maximum (>3 000 µmol m^{-2} s^{-1}). Initial (F_0), maximum (F_m), and variable ($F_v = F_m - F_0$) fluorescence were recorded. F_v/F_m was used to indicate potential maximal quantum yield of PS 2. F_v/F_0 was used to assess PS 2 activity. The area over the fluorescence curve between F_0 and F_m was also recorded and the relative pool size of plastoquinone was estimated from the ratio of this area to the value of $F_m - F_0$. Chl of leaf sample at 45 DAP was extracted with 80 % acetone in the dark for 48 h at 25 °C until leaves were blanched. Absorbances of the clear extract at 652.0, 665.2, and 750 nm were recorded and concentrations of chlorophylls *a*, *b*, and *a* + *b* were computed as described by Porra *et al*. (1984). Plants were harvested at 60 DAP and divided into shoots and roots. Soils were washed out of the roots. These plant samples were rinsed using distilled water, killed at 105 °C for 30 min, dried at 65 - 70 °C for 2 d, and weighed.

The experiment was set up in a completely randomised factorial design (2 Zn treatments × 2 water levels × 3 replicates). All data were subjected to analysis of variance (*ANOVA*), including separation of main effects of Zn supply and soil moisture treatments, and their interactive effects. Means were compared using the Duncan test at the 5 % probability level.

Under well-watered condition, applying Zn increased dry mass of shoots and total plants by 78 and 52 %, respectively. However plant biomass did not respond to Zn addition under dry condition. The shoot growth of Zn treated plants was significantly inhibited by soil water shortage, while root biomass did not change (Table 1). Zn content in shoots was lower than in roots. Zn application increased Zn contents in roots and shoots and in shoots more under water stress (Table 1). Zn application had no obvious effect on the leaf water potential and relative water content (Table 1).

Under well-watered condition, Zn deficiency strongly reduced the photosynthetic performance in maize leaves. P_N, E, and g_s in the Zn deficient leaves decreased by 83, 68, and 74 %, respectively, in comparison to Zn_5 treatment. The water use efficiency, as assessed by ratio of P_N to E, was enhanced markedly by Zn application. However, E and g_s did not alter with exogenous Zn supply under water stress (Table 1).

Zn did not affect Chl *b* and total Chl contents in drought-stressed leaves, while caused increase of Chl *b* and total Chl contents in well-watered leaves. Chl *a* contents in leaves did not change. Chl *a*/*b* ratio was increased by Zn treatment (Table 1).

F_v and F_m of Zn_0 leaves were lower than that of Zn_5 leaves irrespective of soil water supply. F_0 in water-stressed leaves was reduced by Zn deficiency. F_v/F_m was not altered by water stress, implying that the maximum quantum yield of PS 2 was not affected by drought. F_v/F_0 and F_v/F_m were increased by Zn application whether plants were drought stressed or not, suggesting that PS 2 activity and quantum yield were enhanced by Zn. The relative pool size of PQ, calculated as the ratio of the area above the fluorescence induction curve to ($F_m - F_0$), was not influenced by water stress and Zn (Table 1).

Zn deficiency of plants commonly occurs in the arid and semi-arid regions. Ekiz *et al*. (1998) found that wheat yield reduction in Zn deficient calcareous soil was more severe under rain-fed than irrigated conditions. Grewal and Williams (2000) reported alleviation of drought stress by Zn in alfalfa. Bagci *et al*. (2007) observed that the effect of irrigation on grain yield of wheat was maximized when Zn was adequately supplied. The present research done in maize cultivar Zhongdan 9409 showed that Zn had no significant effects on the relative water content and water potential of leaves. Zn addition increased

Table 1. Dry mass, Zn content, leaf water potential, RWC, Chl contents, P_N, E, g_s and parameters derived from chlorophyll fluorescence of maize plants influenced by Zn supply and soil moisture conditions.

Parameters	Well-watered		Water-stressed	
	Zn_0	Zn_5	Zn_0	Zn_5
Shoot dry mass [g plant^{-1}]	0.75 a	1.34 b	0.59 a	0.78 a
Root dry mass [g plant^{-1}]	0.37 a	0.37 a	0.32 a	0.27 a
Total plant dry mass [g plant^{-1}]	1.13 a	1.71 b	0.91 a	1.05 a
Zn content in roots [μg g^{-1}(f.m.)]	29.6 a	48.0 b	28.4 a	44.0 b
Zn content in shoots [μg g^{-1}(f.m.)]	16.1 a	27.2 b	14.3 a	31.7 c
Leaf water potential [-MPa]	0.254 b	0.246 b	1.232 a	1.242 a
RWC [%]	87.1 b	86.8 b	81.4 a	81.1 a
Chl *a* [g kg^{-1}(f.m.)]	1.54 a	1.58 a	1.56 a	1.56 a
Chl *b* [g kg^{-1}(f.m.)]	0.59 a	0.67 b	0.59 a	0.60 a
Chl *a*+*b* [g kg^{-1}(f.m.)]	2.13 a	2.25 b	2.14 a	2.17 a
Chl *a/b*	2.60 b	2.37 a	2.68 c	2.58 b
P_N [μmol m^{-2} s^{-1}]	1.74 b	10.16 c	0.77 a	1.36 ab
E [mmol m^{-2} s^{-1}]	0.50 b	1.55 c	0.32 a	0.35 a
$P_N/E \times 10^{-3}$	3.45 a	6.57 b	2.53 a	3.89 a
g_s [mmol m^{-2} s^{-1}]	13.77 a	52.92 b	9.55 a	9.72 a
F_0	601.8 ab	629.4 b	576.8 a	682.8 c
F_v	1951.0 b	2633.6 c	1127.3 a	2523.8 c
F_m	2552.8 b	3263.0 c	1704.2 a	3206.5 c
F_v/F_m	0.76 ab	0.81 c	0.65 a	0.79 bc
F_v/F_0	3.24 b	4.19 c	1.95 a	3.70 bc
T_m [ms]	268.8 a	284.4 a	350.0 ab	460.8 b
Relative PQ pool	23.9 a	24.5 a	21.7 a	28.3 a

significantly plant biomass under the conditions of well water supply rather than during water stress. A greater reduction of biomass caused by water stress was in plants with Zn fertilization in comparison to Zn deficiency, suggesting that Zn should apply to maize plants grown in Zn deficient soils if they were irrigated, otherwise Zn shortage would decrease plant water use. Similar results were revealed by Khan *et al*. (2003, 2004) who found chickpea yield losses from water stress were greatest at the highest level of Zn. They attributed possible reasons to the limited soil volume afforded by the pots and the rapid development of stress in the larger plants grown at adequate levels of Zn.

Ekiz *et al*. (1998) suggested that lower diffusion of Zn in dry soil under rain-fed conditions restricted root Zn uptake and thus limit plant growth. But our experiment showed that adequate moisture supply did not increase Zn content in maize plants grown in Zn deficient soil. Zn content in those maize plants was still below the generally regarded critical level (about 20 μg g^{-1}) (Liu *et al*.1993, Liu 1996, Marschner 1995).

The present study showed that drought stress inhibited P_N in maize plant. However, applying Zn did not significantly affect P_N in drought stressed plants. Chl fluorescence yield is a sensitive indicator of changes in thylakoid membrane integrity caused by environmental stresses (Bukhov 2004, Weng *et al*. 2008). Adequate Zn nutrition has protective effects on photoxidative damage catalysed by reactive oxygen species in chloroplasts (Cakmak 2000, Wang and Jin 2005). Zn deprivation is reported to depress PS 2 photochemical activity (Balakrishnan *et al*. 2000). In our experiments, Zn application increased the maximum quantum yield and the activity of PS 2, but these did not lead to improvement of P_N in drought stressed maize plants. In fact, it has often shown that the ratio of F_v/F_m is not changed in plants by drought stress (Stuhlfauth *et al*. 1990, Tezara *et al*. 1999, Ohashi *et al.* 2006), indicating that the electron transport capacity in plants is very resilient to drought regardless of plant Zn nutrition.

Our result revealed that Zn addition enhanced P_N of well-watered maize plants which might be associated with increased g_s and so the increase in intercellular CO_2 concentration as suggested by Sharma *et al*. (1994). Zn was possible involved in maintaining high K^+ content in guard cells associated with stomatal opening (Sharma *et al*. 1994, 1995). Zn increased carbonic anhydrase activity might also contribute to the increase of P_N (Ohki 1976, Rengel 1995, Fischer *et al*. 1997, Cakmak and Engels 1999, Hacisalihoglu *et al*. 2003).

In conclusion, positive effects of Zn application on photosynthesis and growth were mostly found under sufficient moisture supply and not under water stress. Therefore, it is better to apply Zn to maize plants grown in Zn deficient soils if they were irrigated.

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