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Stomatal conductance is the main limitation to photosynthesis in sugar beet plants treated with Zn excess

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Abstract:

The effects of high Zn concentrations in growth and photosynthetic parameters of sugar beet (Beta vulgaris L.) plants grown in hydroponics were investigated. Zinc toxicity (100 and 300 µM) resulted in large reductions in biomass accumulation (>50%) and photosynthetic rates (40-50%). It was known that high Zn concentrations usually lead to decreases in net photosynthesis, but the effects of excess Zn on each of the possible factors limiting photosynthesis, including photochemistry, stomatal conductance and mesophyll conductance, had not been studied in detail until now. Leaf photochemistry was not affected by Zn, and reduced photosynthesis was due mostly to marked decreases in stomatal conductance, whereas mesophyll conductance decreased to a lower extent. Stomata were study using scanning electron microscopy (SEM) and large structural differences were observed in stomatal frequency, size and shape between 300 µM Zn and control plants. In high Zn plants stomatal density was lower than in the controls, and stomata were smaller and had a more rounded shape than those present in control plants. The presence of these morphological changes could be associated to the reductions in photosynthetic rates under excess Zn.

Introduction

In low pH soils Zn availability is generally high, and Zn concentrations within the plant could become toxic (Broadley *et al.,* 2007). Excess Zn causes changes in root growth and morphology (Vaillant *et al.*, 2005), reductions in growth (Sagardoy *et al.*, 2009) and decreases in stomatal aperture (Sharma *et al.,* 1995; Sagardoy *et al.*, 2009), whereas photochemistry is not markedly affected (Sagardoy *et al.*, 2009). An increased mesophyll resistance to CO_2 diffusion with Zn toxicity has been also suggested to occur (Van Assche *et al.*, 1980; Prasad and Strzalka, 1999); however, these studies did not use an adequate method to estimate mesophyll diffusion conductance to CO_2 (g_m), but assessed instead a parameter which reflects the combination of g_m and carboxylation activity.

The aim of this work was to investigate the changes in photosynthetic characteristics under excess Zn in the model plant sugar beet (*Beta vulgaris* L.), considering the potential photosynthetic limiting factors mentioned above.

Materials and Methods

Plant material and growth conditions

Sugar beet (*Beta vulgaris* L. Orbis) seeds were germinated and grown in vermiculite for 3 weeks in a growth chamber at 25ºC and a PPFD of 600 µmol m^{-2} s^{-1} with a 16 h light: 8 h dark regime. Afterwards, plants were moved to glasshouse and transplanted to 20-L plastic buckets containing half-Hoagland nutrient solution with 45 µm Fe. Treatments were 1.20 (control), 100 and 300 µM $ZnSO_4$. Plants were used for measurements 10-14 days after imposing the Zn treatments.

Growth parameters and mineral nutrient analysis

Plants were collected and separated in roots and shoots at 14 days. Fresh (FW) and dry weights (DW) of each fraction were measured, and water content (WC) was determined as (fresh weight – dry weight) / fresh weight. Leaf areas were measured with a AM-100 leaf area meter (ADC, Herts, U.K.). Eight plants per treatment were measured. Zinc was determined by FAAS (Igartua *et al.*, 2000).

Gas-exchange and chlorophyll fluorescence measurements

Leaf gas exchange and chlorophyll fluorescence parameters were measured simultaneously using an open gas exchange system (Li-6400; Li-Cor, Inc., Lincoln, NE, USA) with an integrated fluorescence chamber head (Li-6400-40 leaf chamber fluorometer; Li-Cor, Inc.), according to Flexas *et al.* (2007). These measurements were carried out at days 10 to 13 after the start of the treatments, at 1500 µmol m^{-2} s^{-1} with 10% blue light. Cuvette CO_2 concentration (C_a) was set at 400 µmol CO_2 mol^{-1} air and vapor pressure deficit was kept at 2.0 ± 0.2 kPa.

Scanning electron microscopy (SEM-EMX)

The surface of fresh sugar beet leaves was visualized with an Hitachi S-3400 N microscope fitted with an EDX analyzer (Röntec XFlash Si(Li)) at the ICB-CSIC.

Results

Plant dry mass was progressively reduced with high Zn concentrations in the nutrient solution. Plants treated with high Zn contained less water and had smaller leaf areas than control plants. Zinc concentration increased significantly with 100 or 300 µM Zn in the nutrient solution, approximately 10-fold in shoots and 1.6-fold in roots (Table 1).

Zn treatment	**1.2 µM**	**100 µM**	**300 µM**
DW (g plant^{-1})	2.8 ± 0.4^{a}	1.3 ± 0.2^{b}	0.8 ± 0.2^{b}
leaf area (cm^{2})	136.7 ± 5.5^{a}	48.3 ± 5.2^{b}	29.8 ± 3.0^{c}
WC (%)	96.8 ± 0.2^{a}	91.1 ± 0.1^{b}	87.6 ± 0.3^{c}
Zn in shoots (µg g^{-1} DW)	129.7 ± 11.9^{a}	1223.7 ± 64.5^{b}	1184.3 ± 102.7^{b}
Zn in roots (µg g^{-1} DW)	136.1 ± 15.0^{a}	218.4 ± 2.9^{b}	202.9 ± 0.0^{b}

Table 1. *Growth parameters of sugar beet plants grown with different Zn concentrations for 14 days. Data are means ± SE of eight replicates. Data followed by the same letter are not significantly different (Duncan´s test) at p < 0.05 level.*

At normal CO_2 concentration (400 µM CO_2 mol^{-1} air), leaves of plants grown with high concentrations of Zn showed marked decreases in photosynthetic rate when compared to control plants, whereas no significant differences were measured between the 100 and 300 µM Zn treatments (Table 2). Stomatal conductance was drastically reduced (by 70%), whereas mesophyll conductance was reduced less (by 44%). C_i and C_C decreased progressively (Table 2) with increased Zn. No differences were found for maximum carboxylation velocity (V_{max}) and the maximum rate of electron transport (J_{max}) (Table 2), although it was not possible to estimate J_{max} in the 300 µM treatment due to the lack of increase in photosynthetic rates at high CO_2 in these plants.

Zn treatment	**1.2 µM**	**100 µM**	**300 µM**
A_N (µmol CO_2 m^{-2} s^{-1})	21.4 ± 1.2^{a}	12.4 ± 1.4^{b}	11.1 ± 1.8^{b}
F_v/F_m	0.821 ± 0.001^{a}	0.807 ± 0.005^{a}	0.802 ± 0.008^{a}
ETR (µmol e^{-} m^{-2} s^{-1})	143.8 ± 3.1^{a}	114.0 ± 5.8^{a}	120.0 ± 17.3^{a}
Φ_{PSII}	0.218 ± 0.005^{a}	0.173 ± 0.009^{a}	0.182 ± 0.026^{a}
g_s (mol CO_2 m^{-2} s^{-1})	0.231 ± 0.033^{a}	0.070 ± 0.014^{b}	0.055 ± 0.010^{b}
g_m (mol CO_2 m^{-2} s^{-1})	0.389 ± 0.091^{a}	0.243 ± 0.055^{ab}	0.204 ± 0.048^{b}
$V_{c,\ max}$ (µmol CO_2 m^{-2} s^{-1})	104.4 ± 4.5	102.8 ± 5.0	126.5 ± 19.1
J_{max} (µmol CO_2 m^{-2} s^{-1})	128.7 ± 6.7	122.6 ± 1.0	-
C_i (µmol CO_2 mol^{-1} air)	286 ± 8^{a}	200 ± 11^{b}	176 ± 17^{b}
C_c (µmol CO_2 mol^{-1} air)	221 ± 19^{a}	143 ± 14^{b}	115 ± 9^{b}

Table 2. *Photosynthetic parameters measured in intact plants grown in nutrient solution with different concentrations of Zn. Data are means ± SE of five replicates. Data followed by the same letter are not significantly different (Duncan´s test) at p < 0.05 level.*

Leaf surface samples were scanned at 300 x and 3000 x. Zinc-stressed plants had a smoother surface, with smaller, less abundant stomata when compared to the controls (Fig. 1a, b). Stomata in the 300 µM Zn treated plants had a more rounded shape, with stomatal slits shorter than those found in the controls (Fig. 1c, d).

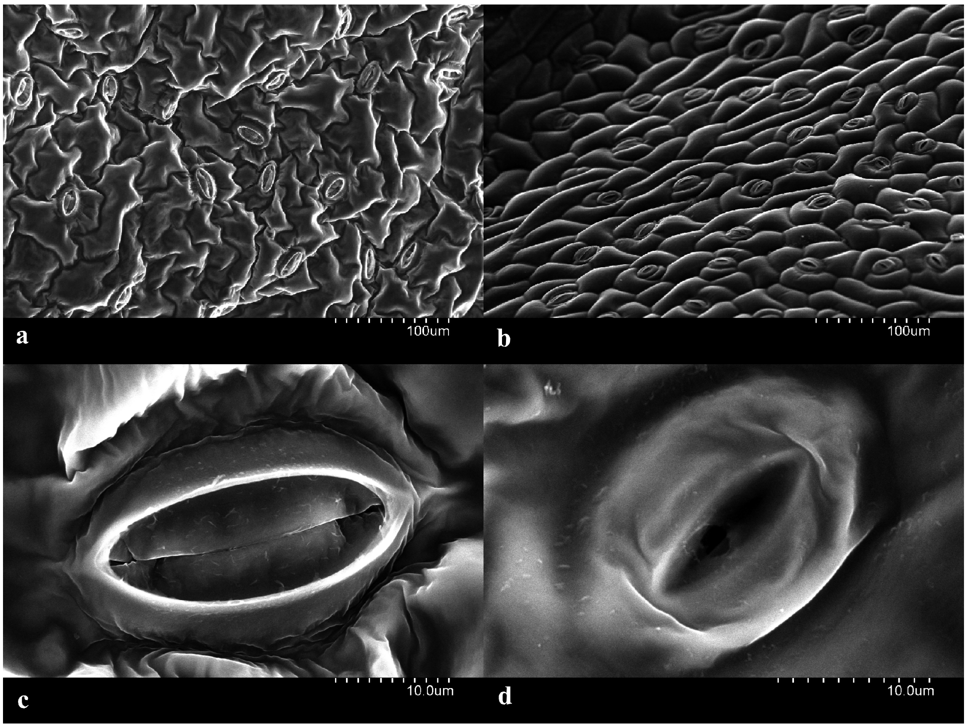

Fig. 1 *Leaf surface (300 x) and stomata (3000 x) from control (a, c) and 300 μM Zn grown sugar beet (b, d) observed with scanning electron microscopy (SEM).*

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