Plant Stress Physiology

Effects of Zn and Cd toxicity on metal concentrations in the xylem sap of *Beta vulgaris* and *Lycopersicon esculentum*

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INTRODUCTION

Zn [1] and Cd [2, 3] toxicities reduce plant growth, chlorophyll and carotenoids concentrations and photosynthetic characteristics, and increase roots Krebs cycle enzyme activities of sugar beet (*Beta vulgaris* L.) and tomato (*Lycopersicon esculentum*) plants grown in hydroponics. An additional physiological effect of these toxicities is an alteration of micronutrient homeostasis. Fe-deficiency was induced by slight and intermediate Zn and Cd toxicities in both species [2, 3], which could be partially due to a failure of the Fe long-distance transport. The aim was to study xylem sap Fe, Zn and Cd concentrations changes in sugar beet and tomato plants grown under different Zn and Cd toxicity levels.

MATERIALS AND METHODS

PLANT MATERIAL

Sugar beet (cv. 'Orbis') and tomato (cv. 'Tres Cantos') seeds were germinated and grown on a growth chamber in hydroponics with ½ Hoagland nutrient solution. Four-week old sugar beet plants were treated with 1.2 µM $ZnSO_4$ (Control), 10 and 50 µM Zn and 10 and 50 µM $CdCl_2$. Three-week old tomato plants were treated with 0 (Control), 10 and 50 µM $CdCl_2$. Treatments were applied for 4 and 8 days and then plants were sampled for xylem sap.

XYLEM SAP COLLECTION

Sugar beet xylem sap was isolated from petioles by excision and subsequent centrifugation for 15 min at 4,000 g and 4 °C [4]. Tomato xylem sap was obtained by pooling the fluid bled for 30 minutes after stem decapitation [5]. Malate dehydrogenase (EC 1.1.1.37) activity was always used as cytosolic contamination marker of xylem sap samples [4].

Fe, Zn AND Cd ANALYSIS

Fresh xylem sap samples (5 replications per treatment) were treated in 1% HNO_3 and analyzed for Fe, Zn and Cd concentrations by Inductively Coupled Plasma (ICP) using two different detectors: Optical Emission Spectroscopy (OES) and Mass Spectrometry (MS) for high and low metal concentration determinations, respectively.

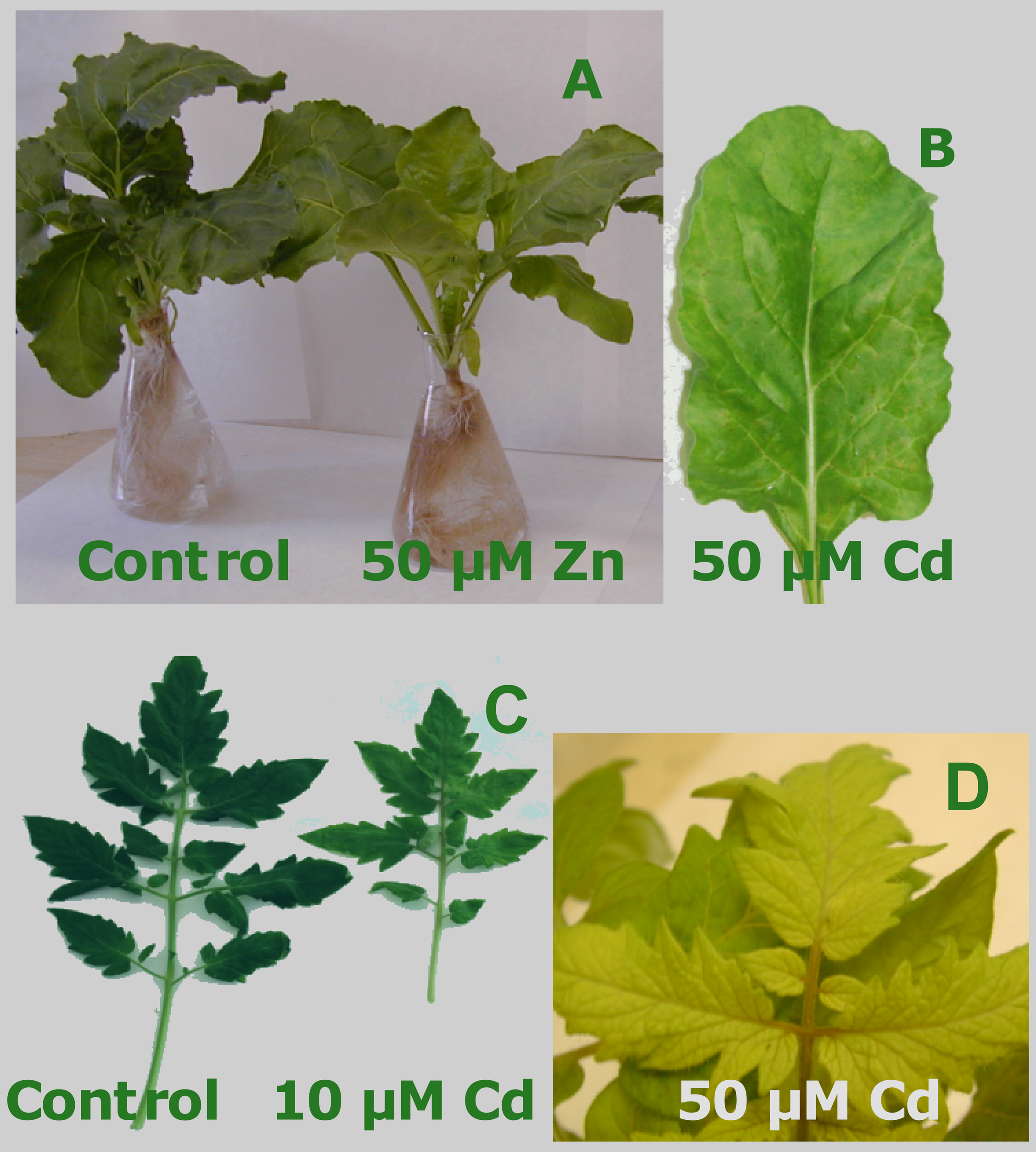

Figure 1. *Toxicity symptoms in sugar beet (A, B) and tomato (C, D) plants.*

RESULTS

Zn TOXICITY: Xylem sap Zn concentrations of plants treated with Zn-excess were between 2- and 8-fold higher than those of control plants. The highest Zn exposure concentrations caused the highest Zn increases and in this case a strong exposure time dependence was observed. Fe concentrations were not affected by Zn treatments.

Cd TOXICITY: All Cd treatments significantly increased xylem Cd concentrations. However, the treatment period differently affected both species. For sugar beet, 8-day of Cd-exposure increased the Cd concentrations approximately 2-fold regarding to those of 4-day Cd-treated plants. Conversely, tomato Cd concentrations of 8-days treated plants were always at least 2-fold lower than those of 4-days treated plants. Fe concentrations were affected by Cd treatments. Sugar beet plants increased Fe transport, whereas tomato plants xylem Fe concentrations decreased with Cd treatments. Zn concentrations were only affected by Cd treatments in tomato plants, increasing at higher Cd treatment levels.

Cd toxicity resulted in large plant-specific alterations of the Fe and Zn long-distance transport but Zn toxicity did not. These data provide the framework for further studies on the identity of the Zn and Cd chemical forms in xylem sap.

Treatment	Treatment days	Fe (µM)	Zn (µM)	Cd (µM)
Sugar beet				
Control		4.53 ± 0.61a	19.18 ± 3.59a	0.04 ± 0.02a
Zn 10 µM	4	8.62 ± 1.23a	58.91 ± 2.07b	-
	8	5.25 ± 0.43a	48.67 ± 4.32b	-
Zn 50 µM	4	6.41 ± 0.74a	90.14 ± 7.95c	0.02 ± 0.00a
	8	6.72 ± 1.73a	148.1 ± 15.6d	0.05 ± 0.00a
Cd 10 µM	4	4.50 ± 0.72a	16.31 ± 1.56a	3.91 ± 0.81b
	8	10.69 ± 1.90c	18.81 ± 3.02a	6.47 ± 1.45bc
Cd 50 µM	4	3.55 ± 0.59a	13.97 ± 1.29a	5.02 ± 0.75b
	8	7.16 ± 0.64b	25.58 ± 6.42a	9.67 ± 1.66c
Tomato				
Control		9.76 ± 1.41b	2.67 ± 2.29a	0.01 ± 0.00a
Cd 10 µM	4	6.07 ± 2.29b	12.17 ± 0.67c	25.09 ± 1.61c
	8	7.58 ± 1.15b	7.35 ± 0.51b	8.66 ± 0.54b
Cd 50 µM	4	1.18 ± 0.20a	9.55 ± 1.08bc	94.63 ± 7.39e
	8	2.50 ± 0.59a	7.10 ± 0.30b	41.86 ± 10.95d

Table 1. *Xylem sap metal concentrations. Data followed by the same letter in the same column and for a given specie are not significantly different at $p < 0.05$. Data are means ± SE (n =5).*

References: [1] Sagardoy *et al.* (2009) Plant Biol 11: 339. [2] Larbi *et al.* (2002) Funct Plant Biol 29: 1453. [3] López-Millán *et al.* (2009) Environ Exp Bot 65: 376. [4] López-Millán *et al.* (2000) Plant Physiol 124: 873. [5] López-Millán *et al.* (2009) J Plant Physiol 166: 375.

Acknowledgements: *This study was supported by the Spanish Ministry of Science and Innovation (Project AGL2007-61948) and the Aragón Government (group A03). R.S. was supported by an I3P-CSIC predoctoral fellowship.*