EFFECTS OF Cd IN THE ROOT PROTEOME OF TOMATO (*Lycopersicon esculentum*) PLANTS

J Rodríguez-Celma, A-F López-Millán, A Abadía and J Abadía

Departamento de Nutrición Vegetal, Estación Experimental de Aula Dei. Zaragoza, CSIC.

Introduction

Heavy metals constitute a heterogeneous group of essential and non-essential elements. Non-essential heavy metals like Cd behave as phytotoxic elements, even when present at low concentrations. Cd accumulation in soils may come from different sources, such as air pollutants and soil applications of commercial fertilizers. In these polluted soils, Cd is generally present as free ions or soluble forms, and it is easily taken up by the roots. Once within the root, Cd is mobilized thoughout the plant, where it can reach edible parts and become a potential hazard for human and animal health. The aim of this work was to investigate the effects of Cd on the root proteomic profile in tomato to further understand the physiological responses of plants to heavy metals.

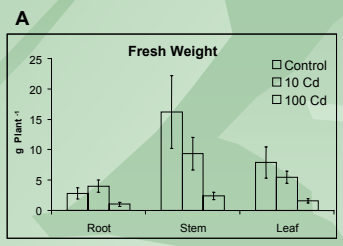
Material and Methods

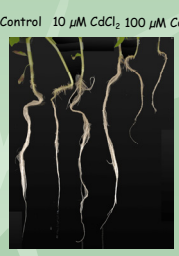
Tomato plants were grown in a controlled environment chamber in hydroponics (80% RH, 23 °C/16h, 18°C/8h, day/night). Cd treatment (10 μM and 100 μM) was imposed 2 weeks after growing plants in control solution and samples were taken 10 days after treatment onset.

2D electrophoresis (IEF-PAGE)

Frozen root tissues were ground in a Retsch MM 301 Mill and proteins extracted with phenol, precipitated and resuspended in rehydration buffer (Urea 8M, CHAPS 2%, DTT 50 mM, PMSF 2 mM, Anpholytes 0.2%). First dimension isoelectric focusing was carried out on 7 cm ReadyStrip IPG Strips (BioRad) with a linear pH gradient from pH 5-8. The second dimension SDS-PAGE was carried out in 12% SDS-polyacrylamide gels, at 20 mAmp per gel for 1.5 h. Gels were subsequently Coomasie-stained and analysed with PDQuest 8.0 software (BioRad). Each experiment was repeated 5 times with 2 plants per batch.

Physiological measurements





Tomato stem and leaf fresh weigths decreased when grown in presence of either 10 or 100 μM Cd, whereas root FW did not change in the 10 μM treatment and decreased in the 100 μM treatment (see A). Leaves showed chlorosis symptoms and necrotic spots and roots acquired a brownish colour (see B).

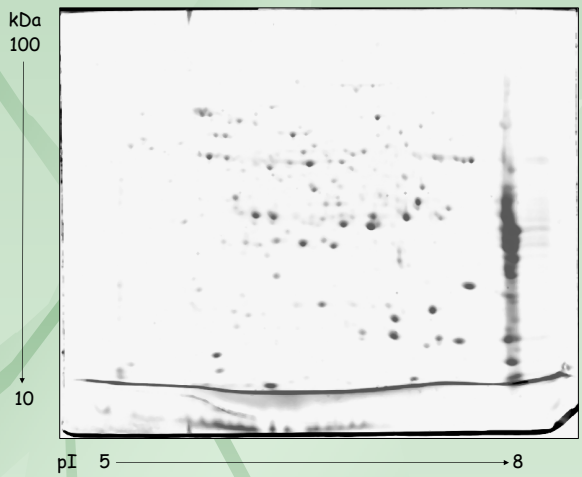
[Cd]	Root	Stem	Leaf
		μg g DW $^{-1}$	
Control	0.67 ± 0.46	0.12 ± 0.04	0.28 ± 0.24
10 μM Cd	1607.26 ± 679.37	152.16 ± 137.13	183.74 ± 54.33
100 μM Cd	4731.44 ± 1323.15	1370.41 ± 338.33	1074.84± 357.80

Cd concentrations in roots, stems and leaves.

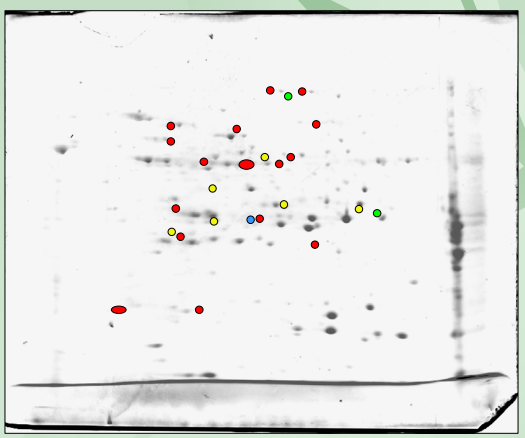
All plant tissues were washed with distilled water. Samples were dried in an oven at 60°C for 72 h until constant weight. For Cd analysis, samples were digested with HNO_3 and hydrogen peroxide (0.1 g in 8 mL HNO_3 and 2 mL H_2O_2) in a microwave system. The digested material was diluted to 25 mL in MilliQ-water and mineral elemental analysis was performed by optical inductively coupled plasma (ICP) spectrometry.

Results and conclusions

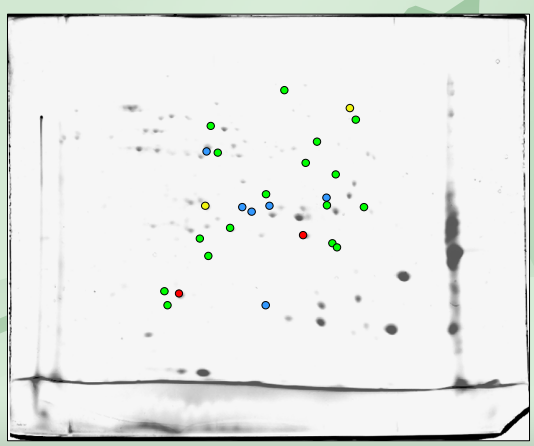
Control



10 μM $CdCl_2$



100 μM $CdCl_2$



Two dimensional separation of root proteins from plants grown with 0, 10, 100 μM Cd resolved 194, 193 and 162 spots, respectively. Averaged polypeptide maps analysis indicated that the 10 μM Cd treatment caused increases in signal intensity in 16 spots and decreases in 2 spots when compared to control plants. Also, 6 and 1 spots were only detected in plants grown with 10 and 0 μM Cd, respectively. When analyzing plants grown with 100 μM Cd, 2 and 18 spots increased and decreased their signal intensity, and 2 and 6 spots were only detected in the 100 and 0 μM Cd grown plants, respectively. From the spots whose intensity changed with Cd supply, 2 spots increased their signal intensity in both 10 and 100 μM Cd treatment, while 2 spots decreased in both.

Conclusion

Cd toxicity induces significant changes in tomato development and root proteome. Further investigation is needed in order to identify the spots that showed changes in intensity with Cd supply and thus better understand plant physiological responses to Cd toxicity.

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