Effects of Cd in the root proteome of tomato plants

Rodríguez-Celma J, López-Millán AF, Abadía A, Abadía J.

Departamento de Nutrición Vegetal, Estación Experimental Aula Dei (CSIC), Apdo. 202, Zaragoza, E-50080.

Introduction

Heavy metals constitute a heterogeneous group of essential and non-essential elements. Non-essential heavy metals such as Cd behave as phytotoxic elements, even when present at low concentrations (Vázquez et al., 1992). Cd accumulation in soils may come from different sources, such as air pollutants and soil applications of commercial fertilizers. When present, Cd is easily taken up by plant roots and mobilized throughout 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

- *Plant Culture:* Tomato (*Lycopersicon esculentum*) plants were grown as described in Zouari *et al*., 2001. Nutrient Solution was supplied with 0, 10, or 100 μM $CdCl_2$.
- *Growth Parameters*: Ten days after treatment onset plants were harvested. Each plant was divided in leaves, stems, and roots, fresh and dry weights were recorded, and ca. 1 g root samples were frozen in liquid N_2. Cd concentration in plant tissues was determined by ICP after tissue digestion in a microwave system.
- *Protein extraction*: Frozen root tissues were ground in a Retsch M301 mill and proteins were extracted with phenol, precipitated and resuspended in rehydration buffer (Meyer et al., 1988)
- *2D electrophoresis*: A first dimension IEF separation was carried out on 7 cm ReadyStrip IPG Strips (pH 5-8; BioRad), with a linear pH gradient in a PROTEAN IEF Cell (BioRad). SDS-PAGE (12% polyacrylamide) was carried out at 20 mA per gel for 1.5 h, and gels were subsequently stained with Commassie-blue and analysed with the PDQuest 8.0 program (BioRad). Experiment was repeated 5 times with 2 different plants per batch.

Results

Cd in nutrient solution decreased root and shoot fresh and dry masses, when compared to control plants. Plants grown with Cd had brownish roots and showed necrotic lesions in the leaf blades. Cd concentrations in roots from plants grown with 0, 10 and 100 μM Cd were 0.7, 1607 and 4731 $\mu g\ g^{-1}$. Two-dimensional separation of root extracts 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 35 spots and decreases in 16 spots, when compared to control plants. Also, 7 and 1 spots were only detected in plants grown with 10 and 0 μM Cd, respectively. When analyzing plants grown with 100 μM Cd, 17 and 47 spots increased and decreased their signal intensity, and 4 and 11 spots were only detected in the 100 and 0 μM Cd grown plants, respectively. From the spots whose intensity changed with Cd supply, 11 spots increased their signal intensity in both 10 and 100 μM Cd treatment, while 7 spots decreased in both.

Conclusions

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.

References

Vázquez, M.D., Poschenrieder, C., Barcelò, J. New Phytol. 1992, 120, 215–226.

Zouari, M., Abadía, A., Abadía, J.J. Plant Nutr. 2001, 24, 1-14.

Meyer, I., Grosset, J., Chartier, Y., Cleyet-Marel J.C. Electrophoresis 1988, 9, 704-712.