

Poster communication

Effects of Cd in the xylem sap of tomato (*Lycopersicon esculentum*) plants: a proteomic approach

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Cd toxicity in crops has become in a serious problem nowadays, specially in developed countries. In soils, Cd accumulation may come from different sources, including air pollutants and soil applications of commercial fertilizers, sewage sludge, manure and lime. In these polluted soils, Cd is generally present as free ions or soluble forms, and its mobility depends on the presence of chelating substances and other cations but being overall easily taken up by the roots. Once within the plant root, Cd is mobilized throughout the plant where it can reach edible parts and become a potential hazard for human and animal health. A critical step in Cd mobilization is xylem sap transport, but little information is still available about this process, including the chemical form(s) in which this heavy metal is present in this fluid. The goal of this work was to study changes induced by Cd toxicity in the proteome of xylem sap obtained from tomato plants, in order to elucidate if any proteins are involved in Cd transport and to better understand the physiological changes involved in Cd toxicity.

Tomato plants cv. Tres Cantos were grown in a controlled environment chamber (80% RH, 23°C-16 h/19°C-8 h day/night regime) in half-strength Hoagland nutrient solution for two weeks. After this period, plants were transferred to nutrient solution containing 0 μM Cd (control) or 10 μM CdCl₂, and grown in these conditions for 10 more days. Xylem sap was obtained by collecting the fluid bled by the plants after stem decapitation. Proteins were precipitated from 10 mL of pooled xylem exudates obtained from 18 plants and resuspended in rehydration buffer (Bio-Rad). First dimension isoelectric focusing was carried out on 7 cm Ready Strip IPG strips (Bio-Rad) with a linear pH gradient from pH 5-8. The second dimension SDS-PAGE was performed in 12% SDS-polyacrilamide gels, at 20 mA per gel for 1.5 h. Gels were subsequently silver stained and analysed with PDQuest 7.1 software (Bio-Rad). In a preliminary study, 13 protein spots of interest were excised from the gels, in-gel digested by trypsin and the mass spectra obtained with a MALDI/TOF-MS apparatus (Bruker Daltonics). The experiment was repeated 3 times.

Two-dimensional separation of xylem sap proteins from plants grown with 0 or 10 μM Cd resolved 204 and 209 spots, respectively. Averaged polypeptide maps analysis indicated that the 10 μM Cd treatment caused increases in signal intensity in 34 spots and decreases in 36 spots, when compared to control plants. Also, 10 and 2 spots were only detected in plants grown with 10 and 0 μM Cd, respectively. The initial batch of spots analyzed included 6 spots exhibiting signal increases and 7 newly detected spots in gels from xylem sap of Cd treated tomatoes. Six spots gave significant matches to known proteins from different species. These proteins include: 3 chitinases: a putative basal resistance related chitinase from *Nicotiana tabacum* (CAI54289), a class II chitinase (AAB96340) from *Solanum tuberosum* and a chitinase (CAA78845) from *Lycopersicon esculentum*, 2 peroxidases: one peroxidase (CAB67121) and a peroxidase precursor (CAA64413) from *Lycopersicon esculentum* and an osmotin 81 (AAP14938) from *Solanum tuberosum*.