EFFECTS OF Cd IN THE XYLEM SAP OF TOMATO (*Lycopersicon esculentum*) PLANTS: A PROTEOMIC APPROACH

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Introduction

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, although its mobility depends on the presence of chelating substances and other cations but it is 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 plant fluid. The goal of this work was to study changes induced by Cd toxicity in the proteome of tomato xylem sap, with the particular aim to identify putative protein targets for Cd toxicity as well as to elucidate if any proteins are involved in Cd transport.

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) was imposed 2 weeks after growing plants in control solution and samples taken 10 days after treatment onset.

Xylem sap samples for analyses were obtained by pooling the fluid bled by 18 plants for 30 minutes after stem decapitation.

Cd concentration

[Cd] in plant tissues was determined by ICP after tissue digestion in a microwave system.

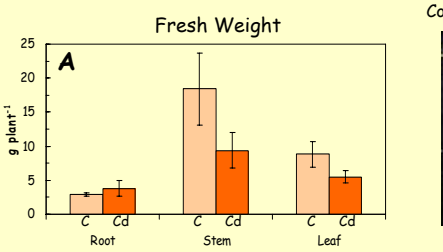
Protein metal affinity purification

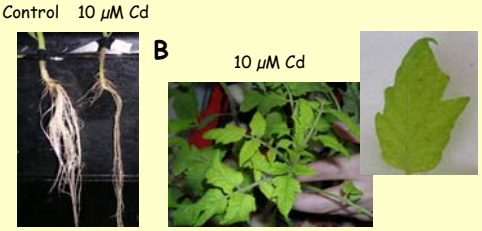
Cd-chelating proteins were separated in a Hi-TRAP chelating column according to the manufacturer's instructions. Proteins in each eluted fractions were separated in SDS-PAGE gels and revealed by silver staining.

2D electrophoresis (IEF-PAGE)/Mass fingerprinting

Proteins were precipitated from 10 mL of pooled xylem exudate and resuspended in rehydratation buffer (Bio-Rad). 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 silver-stained and analysed with PDQuest 8.0 software (BioRad). Protein spots of interest were excised from the gels, in-gel digested by trypsin and mass spectra were obtained with a MALDI/TOF MS apparatus (Bruker Daltonics).

Physiological measurements



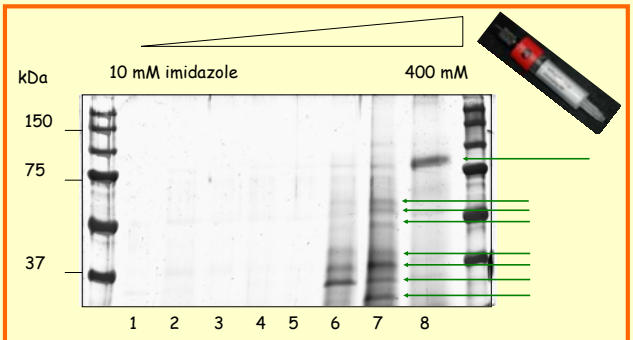


Tomato stem and leaf fresh (see A) and dry (not shown) weights decreased when grown in presence of Cd whereas root fresh (see A) and dry (not shown) weights did not change. Leaves showed chlorosis symptoms and necrotic spots and roots acquired a brownish colour (see B).

[Cd]	Root	Stem	Leaf	Xylem
	Cd, μg g DW^{-1}			Cd, μM
Control	0.67 ± 0.46	0.12 ± 0.04	0.28 ± 0.24	0.01
10 μM Cd	1607 ± 679	152 ± 137	184 ± 54	18.22

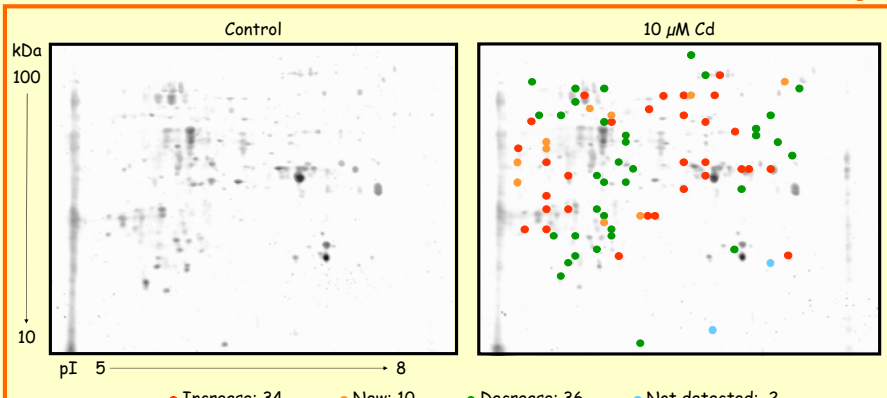
Cd concentrations in roots, stems, leaves and xylem sap.

Cd-affinity chromatography

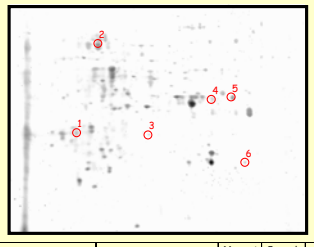


Affinity chromatography with immobilized Cd showed the presence of several proteins strongly retained in the column, which were eluted in the 3 last steps of the elution gradient as revealed by silver staining of the SDS-PAGE gel of the eluted fractions (see above fig.).

2d-IEF/PAGE and mass fingerprinting



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 mM Cd, respectively.



spot	Accession	Homology	Species	Mascot score	Search Limit	MWth (kDa)	Peptide sequence used for identification by MS-MS
1	gi|62719019	putative basal resistance related chitinase	*Nicotiana tabacum*	77	45	11	AINGQIECDGGNPQTVAR
2	gi|6723685	peroxidase	*Lycopersicon esc.*	180	77	35	LGNIGVLTGTNGEIR
3	gi|1236162	class II chitinase	*Solanum tuber.*	117	77	26	GPIQLTHQSNYER
4	gi|19191	chitinase	*Lycopersicon esc.*	126	77	35	WQPSGADQAANR
5	gi|1161566	peroxidase precursor	*Lycopersicon esc.*	123	77	36	SHFQSDPTVAPGLLR
6	gi|30145505	osmotin 81	*Solanum tuber.*	144	77	20	GQTWVINAPR

An initial batch of spots analyzed by MALDI-MS and MS-MS included 34 spots exhibiting signal increases and 7 newly detected spots in gels from xylem sap of Cd treated tomatoes. In this table we show results from six spots which gave significant matches to known proteins from different species (see above figure for localization in the gel).

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