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Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics

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ABSTRACT

The effects of Cd have been investigated in tomato (*Lycopersicon esculentum*) plants grown in a controlled environment in hydroponics, using Cd concentrations of 10 and 100 μM. Cadmium treatment led to major effects in shoots and roots of tomato. Plant growth was reduced in both Cd treatments, leaves showed chlorosis symptoms when grown at 10 μM Cd and necrotic spots when grown at 100 μM Cd, and root browning was observed in both treatments. An increase in the activity of phospho*enol*pyruvate carboxylase, involved in anaplerotic fixation of CO_2 into organic acids, was measured in root extracts of Cd-exposed plants. Also, significant increases in the activities of several enzymes from the Krebs cycle were measured in root extracts of tomato plants grown with Cd. In leaf extracts, significant increases in citrate synthase, isocitrate dehydrogenase and malate dehydrogenase activities were also found at 100 μM Cd, whereas fumarase activity decreased. These data suggest that at low Cd supply (10 μM) tomato plants accumulate Cd in roots and this mechanism may be associated to an increased activity in the PEPC–MDH–CS metabolic pathway involved in citric acid synthesis in roots. Also, at low Cd supply some symptoms associated with a moderate Fe deficiency could be observed, whereas at high Cd supply (100 μM) effects on growth overrule any nutrient interaction caused by excess Cd. Cadmium excess also caused alterations on photosynthetic rates, photosynthetic pigment concentrations and chlorophyll fluorescence, as well as in nutrient homeostasis.

1. Introduction

Cadmium toxicity in crops has become in a serious problem, especially in developed countries. Cadmium accumulation in soils may come from different sources, including air pollutants and soil applications of commercial fertilizers, sewage sludge, manure and lime (McGrath et al., 1994; McLaughlin et al., 1996; Adams et al., 2004; Kidd et al., 2007). Also, industrial effluents may contain a wide variety of pollutants depending on the industries involved, and in many cases high concentrations of heavy metals have been reported (Iribar et al., 2000). In polluted soils, Cd is generally present as free ions or different soluble forms, and its mobility depends on pH (Bingham, 1979) and on the presence of chelating substances and other cations (Hardiman and Jacoby, 1984). Plants can accumulate Cd during plant growth, and the accumulation often occurs in edible parts, thus endangering crop yield and quality and becoming a potential hazard for human and animal health. Cadmium is suggested to cause damage even at very low concentrations, and healthy plants may contain Cd levels that are toxic for mammals (Chen et al., 2007). Moreover, it is widely recognized that Cd taken up by plants is the main source of Cd accumulation in food (Mahaffey et al., 1975; Pinot et al., 2000).

Most of the information available about Cd physiology in plants comes from studies with the Cd-hyperaccumulator *Thlapsi caerulescens* (Lombi et al., 2002) and Cd-tolerant plants such as *Arabidopsis halleri* (Weber et al., 2006; Zhao et al., 2006), whereas less information is available in commercial crops such as tomato. It is commonly assumed that Cd, as well as other heavy metals, are taken up by transporters of essential elements, because of the lack of specificity of these proteins. There is evidence that metal transporters from different families such as ZIP and Nramp are able to transport several divalent cations, including Cd (Korshunova et al., 1999; Pence et al., 2000; Thomine et al., 2000). Also, it has been described that a Ca transport pathway could be involved

Abbreviations: BPDS, bathophenanthroline disulphonate; Chl, chlorophyll; C_i, sub-stomatal CO_2 concentration; CS, citrate synthase; DW, dry weight; *E*, transpiration rate; Φ_{PSII} and Φ_{exc}, actual and intrinsic photosystem II efficiencies, respectively; F_o and F'_o, minimal Chl fluorescence yield in the dark or after light adaptation, respectively; F_m and F'_m, maximal Chl fluorescence yield in the dark or after light adaptation, respectively; FR, far-red; F_S, Chl fluorescence at steady-state photosynthesis; F_v and F'_v, $F_m - F_o$ and $F'_m - F'_o$, respectively; F_v/F_m, maximum potential PSII efficiency; FW, fresh weight; g_S, stomatal conductance; ICDH, isocitrate deshydrogenase; MDH, malate deshydrogenase; NPQ, non-photochemical quenching; PAR, photosynthetic active radiation; PEPC, phospho*enol*pyruvate carboxylase; P_N, net CO_2 uptake rate per unit leaf area; PPFD, photosynthetic photon flux density; PSII, photosystem II; qP, photochemical quenching; V + A + Z, violaxanthin + antheraxanthin + zeaxanthin.

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in Cd uptake (Clemens et al., 1998; Perfus-Barbeoch et al., 2002). Cadmium tolerance in plants is thought to involve internal metal detoxification processes, which may be achieved through both cellular and subcellular compartmentation (Vázquez et al., 1992; Küpper et al., 2000; Ma et al., 2005) and/or complexation with cellular ligands such as phytochelatins, organic acids, cisteine and other low molecular weight thiols (Cobbett and Goldsbrough, 2002; Schat et al., 2002; Küpper et al., 2004; Ueno et al., 2005; Hernández-Allica et al., 2006). Although long distance Cd transport also contributes to Cd distribution and accumulation throughout the plant (Petit and van de Geijn, 1978; Herren and Feller, 1997; Cakmak et al., 2000; Chen et al., 2007), little is known about the chemical form(s) in which this heavy metal is present in xylem and phloem saps. Data available suggest that Cd may be associated in the xylem sap with small molecules such as organic acids (Cataldo et al., 1988; Senden and Wolterbeek, 1990).

Physiological effects of Cd toxicity in plants include inhibition of seed germination, major reductions in growth rates (Huang et al., 1974; Lozano-Rodríguez et al., 1997; Larbi et al., 2002), changes in photosynthetic efficiency, respiration and transpiration (Greger and Ögren, 1991; Krupa et al., 1993; Ciscato et al., 1999; Larbi et al., 2002) and alterations in nutrient homeostasis, including a Cd-induced, Fe deficiency (Wallace et al., 1992; Larbi et al., 2002) and changes in Mn, K, Mg and Ca uptake rates (Greger et al., 1991; Larbi et al., 2002; Dong et al., 2006). At the cellular level, Cd toxicity is known to cause alterations such as membrane damage, disruption of electron transport, inhibition/activation of enzymes and interaction with nucleic acids (Chaoui et al., 1997; Geuns et al., 1997; Clijsters et al., 1999; Leon et al., 2002; Chen et al., 2003a). Possible mechanisms by which these disorders are generated are induction of oxidative stress and replacement of elements such as Zn, Fe, and Mn, which are essential cofactors of many enzymes. Accordingly, there are several reports documenting oxidative stress following exposure to high concentrations of Cd (Smeets et al., 2005; Dong et al., 2006; Lin et al., 2007).

The relevance of tomato (*Lycopersicon esculentum*) in human nutrition is increasing, since it is generally considered as a healthy food because of the high content of lycopene and other health-promoting natural compounds. Tomato is a constituent of the Mediterranean diet, and for instance in Spain (the first tomato producer in the UE and the 7th in the world; FAOSTAT database, http://faostat.fao.org/) this species ranks as one of the most important vegetables in terms of planting area and production (122.000 ha and 4,500,000 t/year). A large part of this crop is grown in greenhouses, using special substrates and fertilization techniques involving reutilization of water, therefore implying an increased risk of heavy metal concentration increases (Gil et al., 2004). Therefore, there is a need to study the responses of food crops such as tomato to Cd toxicity. Thus, the aim of this work was to investigate the effects of two Cd concentrations, 10 and 100 μM, on the photosynthetic characteristics, growth, nutrient composition and several enzymatic activities involved in citric acid synthesis in this crop species.

2. Materials and methods

2.1. Plant material

Tomato (*L. esculentum* Mill cv. Tres Cantos) plants were grown in a growth chamber with a photosynthetic photon flux density (PPFD) at leaf height of 350 μmol m^{-2} s^{-2} PAR, 80% relative humidity and at a 16 h–23 °C/8 h–18 °C, day/night regime. Seeds were germinated and grown in vermiculite for two weeks. Seedlings were grown for an additional two-week period in half-strength Hoagland nutrient solution (Terry, 1980) with 45 μM Fe(III)-EDTA, and then transplanted to 10 L plastic buckets (18 plants per bucket) containing half-strength Hoagland nutrient solution with 45 μM Fe(III)-EDTA and different concentrations of Cd. Treatments used were 0, 10 μM and 100 μM $CdCl_2$. Solutions were changed weekly. Plants were used for measurements 10–12 d after imposing Cd treatments. Young, completely expanded leaves were used for photosynthetic measurements and leaves and root tips were used for enzymatic measurements, except for Fe reductase activity that was measured in whole roots.

2.2. Chemical speciation of the nutrient solution

In silico estimations of the concentrations of Cd ionic species in the different nutrient solutions were carried out with MINTEQA2 from Windows (Version 1.50, Allison Geosience Consultants, Flowery Branch, GA, USA and HydroGeoLogic, Inc., Hermdon, VA, USA).

2.3. Growth parameters

Plants were divided into three fractions, leaves, stems and roots. Fresh (FW) and dry (DW) weights of each fraction, root to shoot ratios and water content per unit DW were also determined. The experiment was run with 3 different batches of plants, and 10 samples per treatment were taken for analysis in each experiment.

2.4. Analysis of Cd and mineral nutrient concentrations

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 nitric acid and hydrogen peroxide (0.1 g in 8 mL HNO_3 and 2 mL H_2O_2) in a microwave system (Ethos Plus, Milestone, Bergamo, Italy). The digested material was diluted to 25 mL in MilliQ-water and mineral elemental analysis was carried out by optical inductively coupled plasma (ICP) spectrometry (ICP-OES spectrometer, IRIS Intrepid II XDL, Thermo Electron Corp., Franklin, MA, USA), equipped with a 2000 W RF generator and full wavelength coverage. For analysis of nutrients, samples were dry-ashed and dissolved in HNO_3 and HCl following the A.O.A.C. procedure (Association of Official Analytical Chemists, Washington DC, USA). Calcium (after La addition), Mg, Fe, Mn, Cu and Zn were determined by FAAS, K by FES and P spectrophotometrically by the molybdate-vanadate method (Igartua et al., 2000). Nitrogen was determined with an NA2100 Nitrogen Analyser (ThermoQuest, Milan, Italy). The experiment was run with 3 different batches of plants and 5 samples per treatment were taken for analysis in each experiment.

2.5. Enzyme activities

Extracts for measuring enzyme activities were made by grinding approximately 100 mg FW of root tip material (or 3 leaf disks, 0.96 cm^2 each) in a mortar with 1 mL of extraction buffer containing 30 mM sorbitol, 1% BSA and 1% PVP in 100 mM HEPES–KOH, pH 8.0. The slurry was centrifuged for 15 min at 10,000 × *g* and 4 °C, and the supernatant was collected and analysed immediately. The activities of all enzymes were analysed in 1 mL (final volume) of the corresponding reaction buffer.

Malate dehydrogenase (MDH, L-malate: NAD-oxidoreductase; EC 1.1.1.37) activity was determined as described in Dannel et al. (1995). Citrate synthase (CS, citrate (Si)-synthase; EC 2.3.3.1) was assayed spectrophotometrically according to Srere (1967) by monitoring the reduction of acetyl CoA to CoA with 5-5′-dithio-bis-2-nitrobenzoic (DTNB) acid at 412 nm. Isocitrate dehydrogenase (ICDH, isocitrate dehydrogenase ($NADP^+$); EC 1.1.1.42) and fumarase (fumarate hydratase; EC 4.2.1.2) activities were determined as described in Bergmeyer et al. (1974). Phospho*enol*pyruvate carboxylase (PEPC, EC 4.1.1.31) activity was

measured in a coupled enzymatic assay with MDH with 75 μL of extract in 2 mM phospho*enol*-pyruvate (PEP), 10 mM $NaHCO_3$, 5 mM $MgCl_2$, 0.16 mM NADH, and 100 mM Bicine–HCl, pH 8.5 (Vance et al., 1983). The experiment was run with 4 different batches of plants, and 3 plants per treatment were taken for activity measurements.

2.6. Root iron reductase activity measurements

Iron reductase activity was measured in whole root systems by following the formation of the Fe(II)-$BPDS_3$ complex from Fe(III)-EDTA (Zouari et al., 2001). Root Fe reductase activity was determined in intact plants after 2–3 h of light onset, 10 days after the Cd treatments were imposed. Plants were placed in 200 mL of a solution containing 1 mM MES, pH 5.5, 100 mM BPDS and 100 mM Fe(III)-EDTA in MilliQ water. The reaction was stopped at 30 min by removing the plant from the container, and absorbance readings of the assay solution at 535 nm were taken after centrifugation. Controls were also carried out in the absence of plants to correct for non-enzymatic Fe reduction. The experiment was run with 3 different batches of plants and 3 plants per treatment were taken for activity measurements.

2.7. Photosynthetic pigment analysis

Photosynthetic pigments were extracted with 100% acetone in the presence of Na ascorbate, and extracts were analysed spectrophotometrically. Photosynthetic pigments were also quantified by HPLC (Larbi et al., 2004). The experiment was replicated 3 times and 5 plants per treatment, illuminated for 3–4 h, were taken for pigment quantifications.

2.8. Gas exchange measurements

Measurements were made on attached leaves in the growth chamber with a portable gas exchange system (CIRAS-1; PP Systems, Hertfordshire, UK), using a PLC broad leaf cuvette in closed circuit mode. Transpiration rate (E), stomatal conductance (g_s), net photosynthetic rate (P_N) and sub-stomatal CO_2 concentration (C_i) were recorded during measurements. Experiments were made at ambient CO_2 concentration, 130–170 $\mu mol\ m^{-2}\ s^{-1}$ PPFD, and at the temperature and relative humidity prevailing in the growth chamber. All measurements were taken in leaves illuminated for 3–4 h. The experiment was run with 3 different batches of plants, and 7 plants per treatment were used for the measurements.

2.9. Modulated chlorophyll fluorescence analysis

Modulated Chl fluorescence measurements were made in attached leaves in the growth chamber with a PAM 2000 apparatus (H. Walz, Effeltrich, Germany). F_o (minimal Chl fluorescence yield in the dark) was measured by switching on the modulated light at 0.6 kHz; PPFD was below 0.1 $\mu mol\ photon\ m^{-2}\ s^{-1}$ at leaf surface. F_m (maximal Chl fluorescence yield in the dark) was measured at 20 kHz with a 1 s pulse of 6000 $\mu mol\ photons\ m^{-2}\ s^{-1}$ of white light. The experimental protocol for the analysis of the Chl fluorescence quenching was as in Morales et al., 2000 and references therein. F_o and F'_o (minimal Chl fluorescence yield after light adaptation) were measured in presence of far red (FR) light (7 $\mu mol\ photon\ m^{-2}\ s^{-1}$) to fully oxidize the PSII acceptor side (Belkhodja et al., 1998; Morales et al., 1998; Logan et al., 2007). Dark-adapted, maximum potential PSII efficiency was calculated as F_v/F_m, where F_v is $F_m - F_o$ (Morales et al., 1991; Abadía et al., 1999). Actual (Φ_{PSII}) and intrinsic (Φ_{exc}) PSII efficiency were calculated as $(F'_m - F_s)/F'_m$ and F'_v/F'_m, respectively. Photochemical quenching (qP) was calculated as $(F'_m - F_s)/F'_v$. Non-photochemical quenching (NPQ) was calculated as $(F_m/F'_m) - 1$. Experiments were carried out at ambient CO_2 concentration, 200–250 $\mu mol\ m^{-2}\ s^{-1}$ PPFD, and at the temperature and relative humidity prevailing in the growth chamber. All measurements were taken in leaves illuminated for 3–4 h. The experiment was replicated 3 times and 10 measurements were made per treatment.

3. Results

3.1. In silico chemical speciation

In the Cd treatments, the major Cd chemical species predicted to occur in the nutrient solution was free Cd^{2+}, accounting for 87.8 and 89.8% of total Cd in the 10 and 100 μM treatments, respectively. Approximately 6.3% of total Cd was predicted to occur in the form of $CdSO_4{}^0$ in both treatments. Also, 4.8% and 1.7% of total Cd were predicted to occur as $Cd[EDTA]^{2-}$ in the 10 and 100 μM treatments, respectively. The species $CdCl^+$ was predicted to occur (1.1% of total Cd) only in the 10 μM Cd treatment.

3.2. Effects of Cd on growth

In the 10 μM Cd treatment the average root mass was larger than that found in the controls and the opposite occurred for leaves and stems, although changes were statistically significant only for leaves and stems (Fig. 1). In the 100 μM Cd treatment, however, both FW and DW in all fractions decreased significantly compared to the control values, and decreases were larger for shoot parts, leaf and stem, than for roots (Fig. 1). Also, both Cd concentrations increased the root/shoot ratio (data not shown). Water content was not changed with Cd treatments (Fig. 1).

3.3. Cd concentrations

Cadmium concentrations increased significantly in all plant parts when increasing Cd in the nutrient solution. Cadmium concentrations in control plants were 0.67 ± 0.46, 0.12 ± 0.04 and 0.28 ± 0.24 $\mu g\ g\ DW^{-1}$ in roots, stems and leaves, respectively. With 10 μM Cd, Cd concentrations were 1607 ± 679, 152 ± 137 and 184 ± 54 $\mu g\ g\ DW^{-1}$ in roots, stems and leaves, whereas with 100 μM Cd concentrations were much higher (4731 ± 1323, 1370 ± 338 and 1075 ± 358 $\mu g\ g\ DW^{-1}$ in roots, stems and leaves, respectively). Total Cd amounts extracted per plant were 537 μg in the 10 μM Cd treatment (354, 69 and 114 μg in roots, stems and leaves, respectively) and 604 μg in the 100 μM Cd treatment (237, 205 and 161 μg in roots, stems and leaves, respectively). Therefore, Cd allocation (in percentage of total Cd, for root/stem/leaf) was 66/13/21 and 39/34/27 in the 10 and 100 μM Cd treatments, respectively.

3.4. Effects of Cd on plant mineral concentrations

Cd toxicity altered plant concentrations of several macroelements. Cd toxicity increased root concentrations of Mg only in plants grown with 10 μM Cd, whereas N concentrations decreased progressively with excess Cd in stems and leaves (12% and 25% in plants grown with 10 and 100 μM Cd, respectively, although changes were statistically significant only with 100 μM Cd) (Fig. 2). Potassium concentrations in roots decreased by 25% with both Cd treatments, whereas in leaves only the highest Cd treatment led to a significant 25% decrease (Fig. 2). Calcium and P concentrations did not show significant changes in any plant part with Cd treatments (Fig. 2).

Micronutrient concentrations were also affected by Cd in the nutrient solution (Fig. 3). Iron concentrations increased 2-fold in both roots and stems in plants grown with 100 μM Cd, whereas in

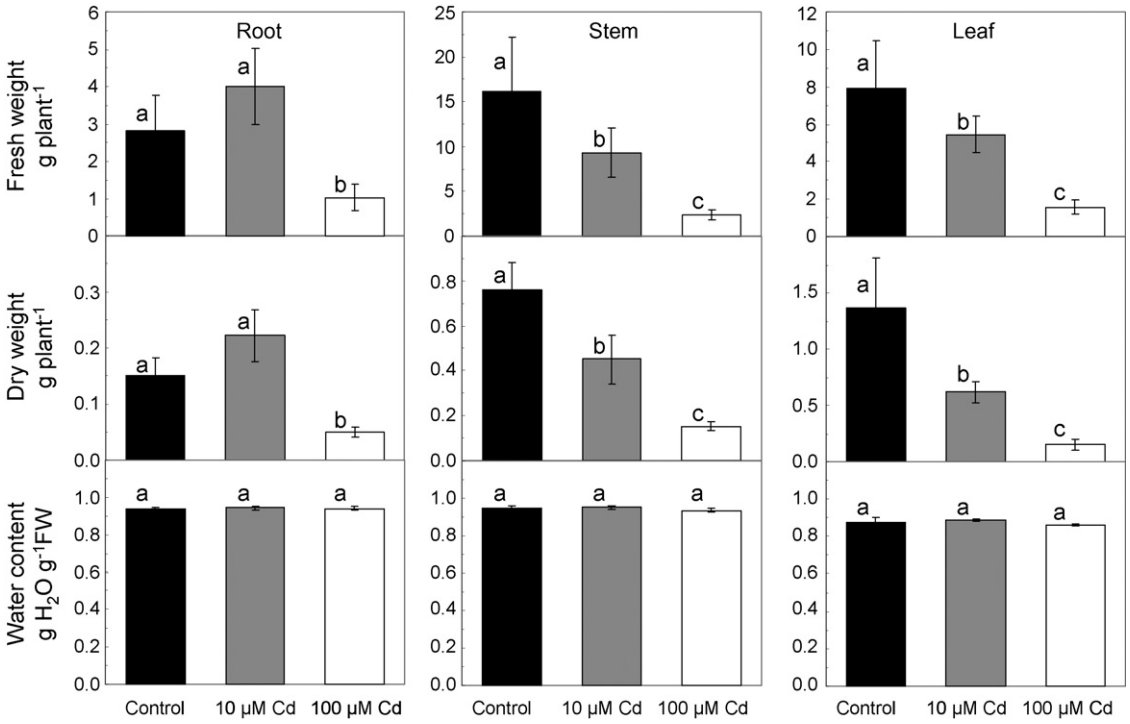

Fig. 1. Fresh and dry masses (in g per plant) and water content (in g g^{-1} FW) of roots, stems and leaves of tomato plants grown in absence (control) and in presence of Cd (10 and 100 μM Cd). Data are means ± SD of 30 replications (3 batches of plants with 10 replicates per treatment). Columns marked with the same letter are not significantly different (Student's test) at the $p < 0.05$ level.

leaves it did not change. No significant differences in Fe concentrations were measured in the 10 μM Cd treatment in roots and stems, but leaf Fe concentration decreased by 50% when compared to controls (Fig. 3). Zinc concentrations also increased (2-fold) in roots of plants grown with 100 μM Cd, but no significant differences were observed in stems and leaves in any of the treatments (Fig. 3). Copper concentrations in roots increased by 50% only with 100 μM Cd supply, whereas in leaves and stems it decreased by 40 and 50%, respectively (Fig. 3). In contrast, Mn concentrations decreased markedly in roots with both Cd treatments, whereas in stems and leaves it did not change significantly (Fig. 3), except for leaves at 10 μM Cd, where there was a slight but significant increase.

3.5. Effects of Cd on enzyme activities

The activities of five enzymes involved in organic acid metabolism in root tip and leaf extracts of tomato plants were measured. In root tip extracts, the activities of ICDH, CS and fumarase increased progressively as Cd concentration in the nutrient solution increased (Table 1). Activities of PEPC and MDH increased 2.7-fold and 1.4-fold respectively, in the 10 μM Cd treatment and no further increases were measured in the 100 μM Cd treatment when compared to controls (Table 1). Leaf extracts from plants grown with 100 μM Cd showed significant increases in the activities of ICDH (4.4-fold), CS (3.7-fold) and MDH (1.7-fold) when compared to activities measured in control leaves (Table 1). The activity of fumarase in extracts from the same leaves decreased by 50% when compared to controls, whereas the activity of PEPC did not change (Table 1). In the 10 μM Cd treatment, changes were not significant except for small increases in CS and MDH (Table 1).

3.6. Effects of Cd on root iron reductase activity

Roots of tomato became brownish when grown with Cd in the nutrient solution. Whole root Fe reductase activities decreased when Cd concentration increased in the nutrient solution. Iron reductase activity in control plants was 0.29 ± 0.06 μmol Fe reduced g^{-1} FW h^{-1}. Activities decreased by 38% (to 0.18 ± 0.03 μmol Fe reduced g^{-1} FW h^{-1}) and 62% (to 0.11 ± 0.03 μmol Fe reduced g^{-1} FW h^{-1}) in the 10 and 100 μM Cd treatments, respectively.

Table 1
Enzymatic activities in extracts of leaves and root tips (in μmol substrate g^{-1} FW min^{-1}) of control and Cd-treated (10 and 100 μM Cd) tomato plants. Data are means ± SE of 12 replications (4 batches of plants with 3 replicates per treatment). Data followed by the same letter within the same column are not significantly different (Student's test) at the $p < 0.05$ level.

Leaves	ICDH	CS	Fumarase	MDH	PEPC
Control	0.20 ± 0.02 a	0.18 ± 0.01 a	315.8 ± 13.4 b	27.38 ± 0.81 a	0.95 ± 0.19 a
10 μM Cd	0.22 ± 0.08 a	0.25 ± 0.05 b	383.3 ± 61.5 b	36.24 ± 7.16 b	1.11 ± 0.26 a
100 μM Cd	0.88 ± 0.06 b	0.66 ± 0.21 c	142.5 ± 20.5 a	45.73 ± 0.81 c	1.15 ± 0.17 a
Roots	**ICDH**	**CS**	**Fumarase**	**MDH**	**PEPC**
Control	0.33 ± 0.03 a	0.05 ± 0.01 a	310 ± 85 a	13.07 ± 2.29 a	0.31 ± 0.10 a
10 μM Cd	0.59 ± 0.08 b	0.23 ± 0.04 b	763 ± 209 b	18.58 ± 1.33 b	0.85 ± 0.09 b
100 μM Cd	0.93 ± 0.11 c	0.42 ± 0.02 c	895 ± 375 b	19.61 ± 2.07 b	0.84 ± 0.09 b

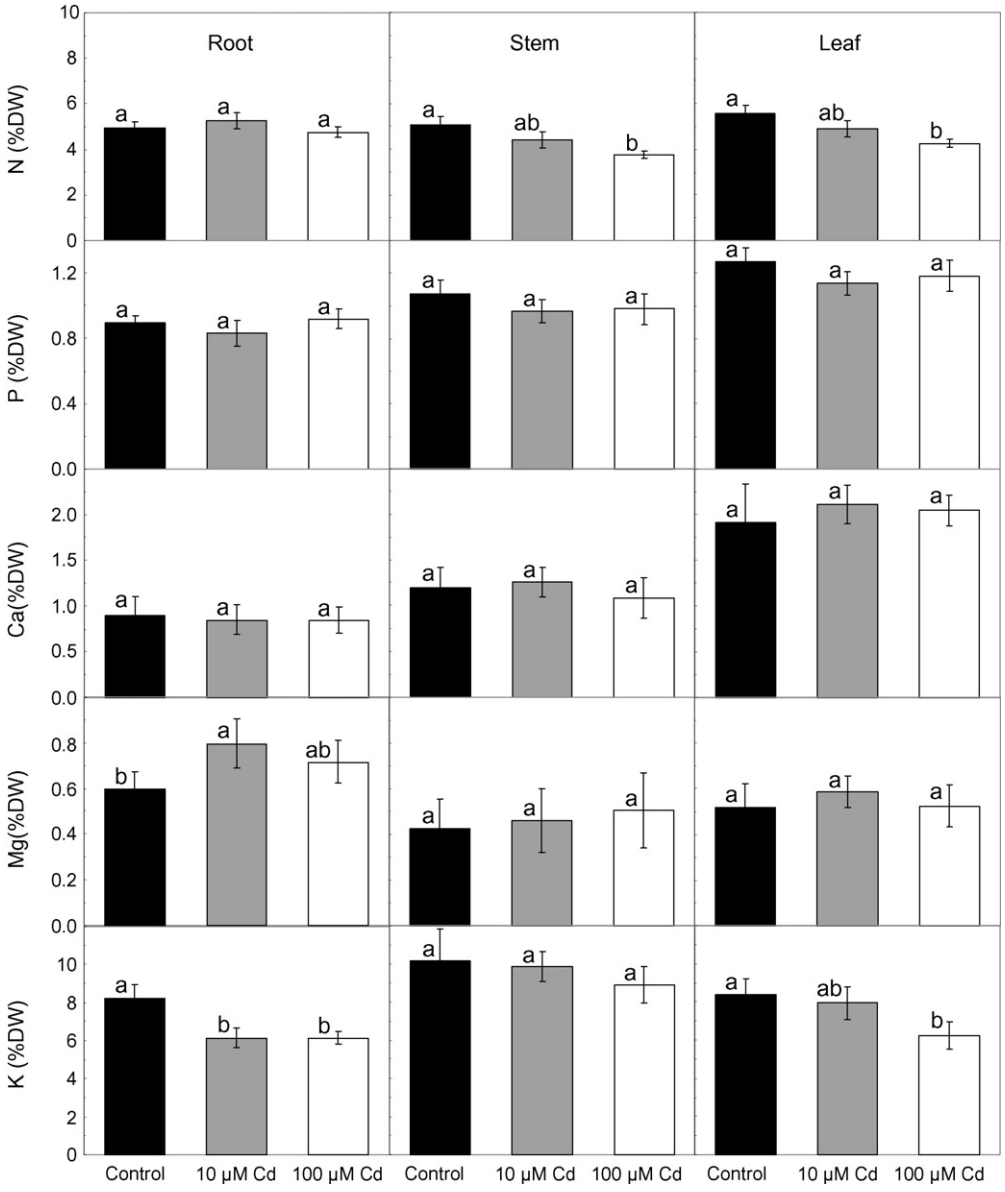

Fig. 2. Macronutrient concentrations (in %DW) in roots, stems and leaves of tomato plants grown in absence (control) and in presence of Cd (10 and 100 μM Cd). Data are means ± SD of 15 replications (3 batches of plants with 5 replicates per treatment). Columns marked with the same letter are not significantly different (Student's test) at the $p < 0.05$ level.

3.7. Effects of Cd on photosynthetic pigment composition

In the 10 μM Cd treatment leaf chlorosis was observed, and accordingly concentrations of all major photosynthetic pigments on a leaf area basis, except for β-carotene, were decreased when compared to those found in control plants (Fig. 4). In leaves of plants grown with 100 μM Cd, no marked chlorosis was observed but leaf size was reduced, moderate decreases in the concentrations of photosynthetic pigments (excepting β-carotene) were found and necrotic spots in the leaf blade were apparent. Leaf pigment decreases were (for the 10/100 μM Cd treatments) 50/44% for neoxanthin, 42/20% for lutein, 39/18% for violaxanthin, 40-35% for Chl *a* and 46–23% for Chl *b*. As a result of these changes, the average Chl *a*/*b* ratio increased in leaves of tomato plants treated with 10 μM Cd to 3.3 from the control values of 3.0, and decreased in those treated with 100 μM Cd to a value of 2.8, although these changes were not statistically significant at $p < 0.05$. The violaxanthin/Chl ratio increased in plants treated with 10 and 100 μM Cd (data not shown). Antheraxanthin and zeaxanthin were not detected in any of the treatments.

3.8. Effects of Cd on gas exchange parameters

Cd treatments reduced P_N and g_s when compared to control plants. Reductions in P_N were 31 and 73% in the 10 and 100 μM treatments, respectively, and reductions in g_s were 17 and 62%, respectively (Fig. 5). When compared to values found in control plants, transpiration rate increased in the 10 μM $CdCl_2$ treatment and decreased by 44% in the 100 μM $CdCl_2$ treatment (Fig. 5). C_i values did not change with Cd treatments (Fig. 5).

3.9. Effects of Cd on chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters including F_v/F_m ratios, actual PSII efficiency (Φ_{PSII}), intrinsic PSII efficiency (Φ_{exc}) and photochemical quenching (qP) did not change significantly in plants

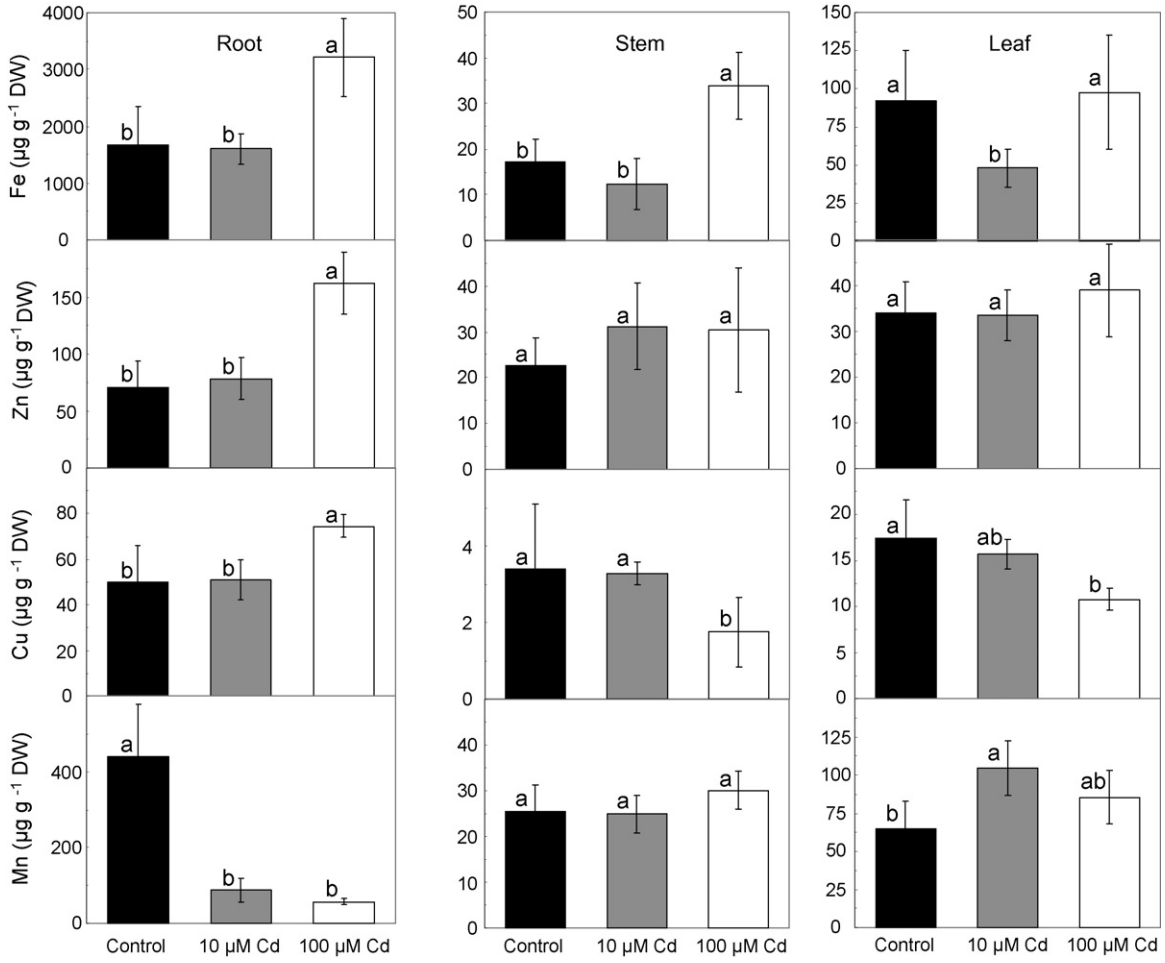

Fig. 3. Micronutrient concentrations (in $\mu g\,g^{-1}$ DW) in roots, stems and leaves of tomato plants grown in absence (control) and in presence of Cd (10 and 100 μM Cd). Data are means ± SD of 15 replications (3 batches of plants with 5 replicates per treatment). Columns marked with the same letter are not significantly different (Student's test) at the $p < 0.05$ level.

Table 2
Modulated chlorophyll fluorescence parameters in control and Cd-treated (10 and 100 μM Cd) tomato plants. The incident PPFD was between 130 and 170 $\mu mol\,m^{-2}\,s^{-1}$. Data are means ± SD of 30 replications (3 batches of plants with 10 replicates per treatment). Data followed by the same letter within the same column are not significantly different (Student's test) at the $p < 0.05$ level.

	F_v/F_m	Φ_{PSII}	Φ_{exc}	qP	NPQ
Control	0.81 ± 0.01 a	0.66 ± 0.05 a	0.68 ± 0.04 a	0.98 ± 0.01 a	0.21 ± 0.01 a
10 μM Cd	0.81 ± 0.01 a	0.62 ± 0.05 a	0.65 ± 0.04 a	0.96 ± 0.02 a	0.25 ± 0.04 ab
100 μM Cd	0.79 ± 0.02 a	0.60 ± 0.08 a	0.62 ± 0.08 a	0.96 ± 0.02 a	0.34 ± 0.10 b

grown with 10 or 100 μM $CdCl_2$ when compared to control values (Table 2). Non-photochemical quenching increased with Cd in the nutrient solution, although this increase was only significant in the 100 μM Cd treatment (Table 2).

4. Discussion

Cadmium treatment led to major effects in shoots and roots of tomato. Plant growth was reduced in both treatments, leaves showed chlorosis symptoms when grown at 10 μM Cd and necrotic spots when grown at 100 μM Cd, and root browning was also observed in both treatments. An increase in the activity of PEPC, involved in anaplerotic fixation of CO_2 into organic acids, was measured in roots of Cd exposed plants. Also, significant increases in the activities of several enzymes from the Krebs cycle were measured in roots of tomato plants grown with Cd. Cadmium excess caused several alterations on photosynthetic rates, photosynthetic pigments and chlorophyll fluorescence as well as in nutrient homeostasis.

Cadmium allocation in tomato plants depended on the Cd concentration in the nutrient solution. In the 10 μM Cd treatment, Cd^{2+} concentration in the nutrient solution was approximately 9 μM and the total amount of Cd extracted per plant was 537 μg. In the much stronger 100 μM Cd treatment, Cd^{2+} concentration in the nutrient solution was approximately 90 μM but the total amount of Cd extracted by plants was only 12% larger than that found with the 10 μM Cd treatment (604 μg per plant). However, Cd allocation was very different in the two treatments, and a major increase in the metal allocated in shoots (from 34% to 61%) was found when Cd in the nutrient solution increased from 10 to 100 μM Cd. Conversely, the metal allocated in roots was 66% in the 10 μM Cd treatment and only 39% in the 100 μM treatment. In control plants less than 1 μg of Cd was taken up, most likely from Cd present in the salts used to build the nutrient solution, and the distribution of this metal was 17, 16 and 67% in roots, stems and leaves, respectively (results not shown). These data indicate that, as it occurs with other plant species, the Cd detoxification strategy in tomato plants grown with

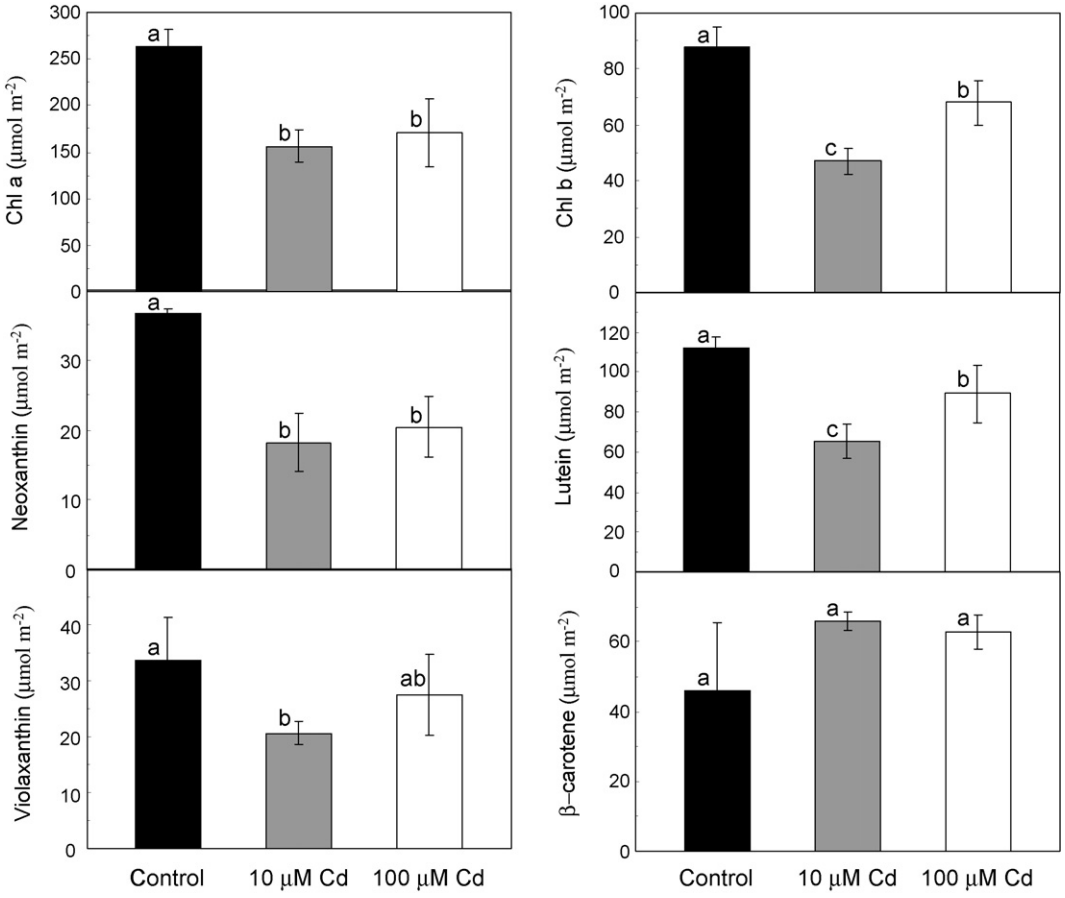

Fig. 4. Leaf concentrations of photosynthetic pigments (carotenoids and chlorophylls, in μmol m^{-2}) in tomato plants grown in absence (control) and in presence of Cd (10 and 100 μM Cd). Data are means ± SD of 15 replications (3 batches of plants with 5 replicates per treatment). Columns marked with the same letter are not significantly different (Student's test) at the $p < 0.05$ level.

low Cd concentrations relies on accumulating Cd at the root level. At higher Cd concentrations, however, roots are overloaded with Cd and a significant mobilization to the shoot occurs.

The fact that total Cd uptake was only slightly larger in the 100 μM Cd treatment (10.8 mg Cd with 18 plants) when compared to that found in the 10 μM Cd (9.7 mg Cd with 18 plants) deserves further investigation. High Cd concentrations in the nutrient solution may lead to a large Cd uptake by the plant in the short-term subsequently impairing further metal uptake. Several explanations such as root Cd re-extrusion to the medium in the 100 μM Cd treatment are possible; however further studies including total Cd uptake data in the midst of the treatment period would be necessary to prove any possible hypotheses.

Cadmium supply increased root activities of PEPC and several enzymes from the Krebs cycle, including MDH, CS, fumarase and ICDH, with CS showing the largest increases (4.6- and 8.4-fold in the 10 and 100 μM Cd treatments, respectively). A coordinated increase in the root activities of PEPC, MDH and CS could cause carboxylation of PEP to OAA, and subsequently lead to malate and citrate, thus acting as an anaplerotic mechanism for CO_2 fixation. The ability to take up Cd in different species has been correlated to differences in the capacity of roots to secrete citric acid (Chen et al., 2003b; Duarte et al., 2007; Liu et al., 2007). Organic acid excretion by roots has also been reported in several nutritional stresses such as P and Fe deficiency, as well as in Al toxicity (Jones, 1998; Abadía et al., 2002; Liao et al., 2006; Kobayashi et al., 2007), and similar increases in the activities of PEPC, MDH and CS have been measured in P- and Fe-deficient roots (Johnson et al., 1994; López-Millán et al., 2000).

In tomato leaves PEPC activity did not increase with Cd supply in any of the treatments, suggesting that anaplerotic fixation of CO_2 is restricted to roots. Although significant increases in leaf activities of CS, MDH and ICDH were measured in the 100 μM Cd supply treatment, fumarase activity decreased. In the 10 μM Cd treatment however, leaf fumarase activity did not decrease and increases in CS, MDH and ICDH were less marked. Fumarase is unique to the mithochondrion and therefore is a convenient marker for the mitochondrial matrix (Siedow and Day, 2000). These results suggest that at high Cd supply leaf mitochondrial activity could be impaired and increases in CS, MDH and ICDH leaf activities may reflect cytoplasmic changes trying to cope with the reduction in citric acid cycle activities. An increase in ICDH has also been reported in leaves of *Phaseolus vulgaris* after Cd application (Smeets et al., 2005). An increased activity of enzymes such as MDH and ICDH can also generate reducing power, which may play a role in redox mechanisms in plant cells. It has been widely described that Cd exposure causes oxidative stress, and accordingly several enzymes and metabolites involved in the plant defence mechanisms against oxidative stress are elicited by Cd (Lozano-Rodríguez et al., 1997; Smeets et al., 2005; Dong et al., 2006; Lin et al., 2007).

Cadmium treatments led to progressive decreases in net CO_2 uptake (P_N). Cadmium induced decreases in the leaf concentrations of chlorophyll and other photosynthetic pigment concentrations, and the effect was more marked in the 10 μM Cd treatment than in the 100 μM one. A similar effect was observed in sugar beet, and attributed to a Cd-induced Fe deficiency at low Cd treatments, where Cd effects on growth were still moderate (Larbi et al., 2002). At higher Cd levels, effects on growth were so strong that the

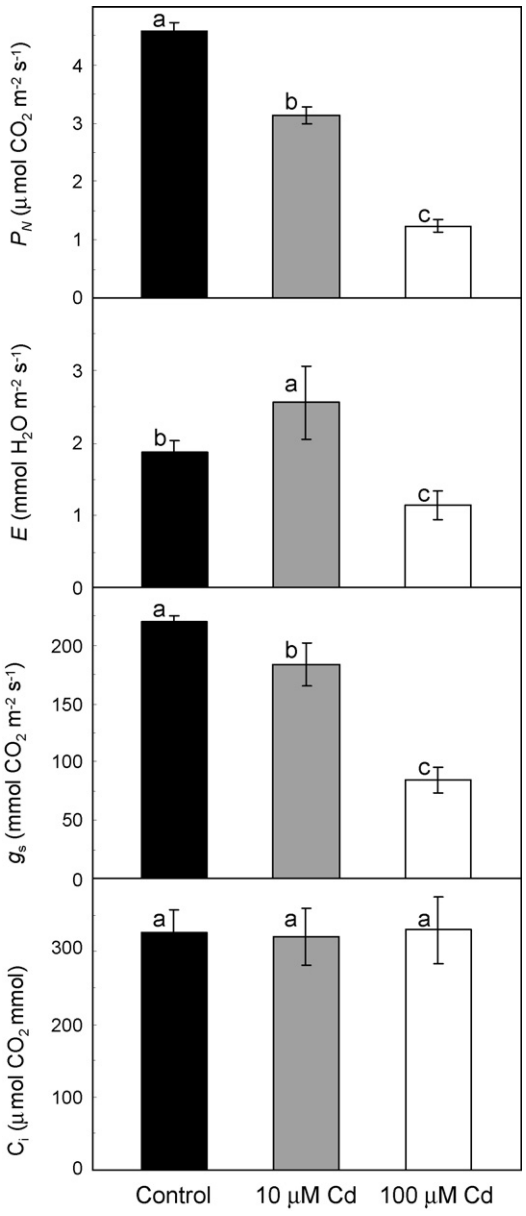

Fig. 5. Gas exchange parameters in leaves of tomato plants grown in absence (control) and in presence of Cd (10 and 100 μM Cd). The incident PPFD was between 130 and 170 μmol m^{-2} s^{-1}. Data are means ± SD of 21 replications (3 batches of plants with 7 replicates per treatment). Columns marked with the same letter are not significantly different (Student's test) at the $p < 0.05$ level.

requirement for Fe was much lower and Fe-deficiency symptoms were no longer evident (Larbi et al., 2002). No de-epoxidation of xanthophyll cycle pigments occurred even when mild leaf chlorosis occurred at 10 μM Cd. The same was observed in sugar beet grown at 50 μM Cd, but not at 10 μM Cd, since at low Cd zeaxanthin and antheraxantin were formed (Larbi et al., 2002). The lack of major effects of Cd on leaf electron transport rates, NPQ and xanthophyll cycle pigments de-epoxidation may support that Cd-treated tomato plants were not affected by photo-inhibitory processes. However, the possibility that the xanthophyll cycle pigments photoprotection mechanism could be inactivated by Cd metal toxicity in tomato (and sugar beet) leaves cannot be ruled out at this stage.

Overall, results suggest that in tomato grown with 10 μM Cd, Cd-induced Fe-deficiency is not the main effect, and that this Cd concentration is enough to lead to direct effects in photosynthesis. However, some signs of a moderate Fe deficiency were present in the 10 μM Cd treatment, including the moderately low leaf Fe concentrations (50 μg g^{-1} DW) and the mild chlorosis symptoms. Root Fe reductase activity, a known sign of Fe-stress, decreased in tomato plants grown with Cd. Heavy metals have been described previously, on one hand, to increase root ferric chelate reductase activity because of the limitations imposed to Fe trafficking (Larbi et al., 2002), and on the other hand, to decrease this activity due to a direct effect on the reductase enzyme itself (Alcántara et al., 1994; Chang et al., 2003). In the case of tomato, the direct effect of Cd apparently overrules any promoting effect associated to the mild Fe deficiency caused by Cd.

Other alterations in micronutrient concentrations were observed in tomato plants grown with Cd. Major changes included increases in root Fe, Zn and Cu concentrations when plants were grown with 100 μM Cd and a progressive decrease in Mn concentration with increased Cd supply. Synergistic effects between high Cd concentrations and Fe, Cu and Zn root accumulation have been described before for other species (Larbi et al., 2002; Liu et al., 2003) and in agreement with our data no such changes were observed in tomato roots with low Cd supply (Dong et al., 2006). It has been hypothesized that increases in root concentrations of divalent metals could be partially explained by Cd interference in nutrient uptake by affecting the permeability of plasma membranes (Dong et al., 2006). Also, the lack of specificity of members of several families of divalent metal transporters such as ZIP and Nramp could contribute to this fact. For instance, a member of the ZIP family, ZIP9, is induced in *Arabidopsis thaliana* roots in presence of Cd and is constitutively highly expressed in the roots of the accumulator *A. halleri* (Weber et al., 2006). Antagonistic effects between high Cd concentrations and Mn uptake and transport have been reported in many studies (Hernández et al., 1998; Larbi et al., 2002; Dong et al., 2006; Wu et al., 2007), and a reduction in Cd uptake in presence of Mn has been observed (Cataldo et al., 1981). Reports are contradictory regarding the influence of Cd in shoot micronutrient concentrations; for instance $CdCl_2$ decreased Zn and Cu shoot concentrations in sugar beet (Larbi et al., 2002) whereas in tomato no differences were found (Dong et al., 2006, and this work). Differences among species may arise from the different micronutrient homeostasis and Cd detoxification mechanisms; for instance, at low Cd sugar beet is able to mobilize more Cd to shoots than tomato (up to 50 and 34% of total Cd, respectively).

In conclusion, at low Cd levels (10 μM), which are possible to occur in intensive horticultural systems, tomato could transport amounts of Cd to the shoot that might become a health hazard, and although decreases in growth and photosynthesis and a mild chlorosis could be noticed quite easily, the detection of Cd excess can only be done through plant tissue Cd analysis. At low Cd supply, tomato plants accumulated Cd in roots and this mechanism may be associated to an increased activity in the PEPC–MDH–CS metabolic pathway via citric acid synthesis by roots. Also, alterations in photosynthesis and photosynthetic pigments may suggest a direct toxic effect of Cd on photosynthesis in addition to a mild Cd-induced Fe deficiency. At high Cd supply (100 μM) effects on growth overrule any nutrient interaction caused by excess Cd.

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