Journal of Biological Research-Thessaloniki **13**: 75 – 84, 2010
J. Biol. Res.-Thessalon. is available online at http://www.jbr.gr
Indexed in: WoS (Web of Science, ISI Thomson), SCOPUS, CAS (Chemical Abstracts Service) and DOAJ (Directory of Open Access Journals)

Aspects on growth and anatomy of internodes and leaves of cadmium-treated *Solanum lycopersicum* L. plants

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Received: 26 January 2009 Accepted after revision: 12 June 2009

The effects of cadmium (Cd) on growth parameters and anatomy of tomato (*Solanum lycopersicum* L.) internodes and leaves differing in age were addressed. Six-day-old seedlings were hydroponically cultivated and exposed for 10 d to increasing concentrations of $CdCl_2$ (0-100 μM). Cd restricted both fresh and dry mass of leaves and internodes depending on the tissue age. Morphological changes were observed following Cd-exposure, including the reduction in the internode length and diameter as well as decreasing leaf area and thickness. Stem morphogenesis was altered too, cellular division disorders being associated with abnormal enlargement of both cortical and medullar cell layers. Histological study at the internode level revealed that Cd restricted the expansion of tissues in concomitance with the presence of peculiar structures in the cortical and medullar parenchymas. Furthermore, under high Cd-treatment (100 μM $CdCl_2$), reduction of the mesophyll cell size and spongy intercellular space volume leading to leaf blade thinning was observed. The intensity of the above Cd-induced anatomical changes differed depending on the organ developmental stages at which they were first exposed to the contaminant.

Key words: anatomy, cadmium, leaf, stem, *Solanum lycopersicum*.

INTRODUCTION

Cadmium is one of the most hazardous heavy metal pollutants released to the environment as a result of anthropogenic activities (Wagner, 1993). Although not essential for plant growth, Cd is readily taken up and accumulated in plants at variable amounts (Koopmans *et al*., 2008). The accumulation of Cd in roots and shoots depends on binding to extracellular matrix (Horst, 1995), complexing inside the cell (Cobbett, 2000) and on the transport efficiency (Marchiol *et al*., 1996). Chlorosis, leaf rolling and stunting are the main and easily visible symptoms of cadmium toxicity in plants. At high concentrations, Cd can be toxic for several species, causing structural disorders and thereby growth restriction (Barcelo *et al*., 1988a, b; Djebali *et al*., 2002, 2005; Zoghlami *et al*., 2006; Jin *et al*., 2008). A number of detrimental effects of Cd on metabolism have also been reported, such as decreased nutrient uptake (Ghnaya *et al*., 2007), changes in nitrogen metabolism (Wang *et al*., 2008), altering of water balance, and inhibition of stomatal opening (Sandalio *et al*., 2001). Moreover, Cd^{2+} ions might cause alterations in permeability of membranes by affecting lipid composition (Djebali *et al*., 2005) and certain enzymes associated with membranes, such as H^+-ATPase (Fodor *et al*., 1995). Net photosynthesis is also sensitive to Cd, which directly affects chlorophyll biosynthesis (Ekmekçi *et al*., 2008; Li *et al*., 2008) and proper development of chloroplasts (Djebali *et al*., 2005; Jin *et al*., 2008).

Despite the achievements in elucidating Cd phytotoxicity, its physiological nature is not fully understood. This is essentially due to (i) the manifold and interlacing effects exerted by the metal to basic e-

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vents of plant growth and development and (ii) to the fact that the responses to Cd, as regards a given event, may vary according to the species. One of the most controversial issues is that refers to the relationship between plant growth inhibition and disorders in the cardinal physiological processes.

Numerous studies have described symptoms and responses of plants upon Cd-exposure (Djebali *et al*., 2002, 2005; Zoghlami *et al*., 2006; Jin *et al*., 2008). Mysterious are the primary targets of toxicity and the degree of the response specificities. Cadmium can be accumulated to high levels in the aerial organs (Pence *et al*., 2000) and considerably alter shoot-leaf structure and metabolism (Siedlecka *et al*., 1997; Jin *et al*., 2008). Whether dicotyledonous or monocotyledonous, C3 or C4, plants respond differently to heavy metal stress, depending on the growth stages at which they were exposed to a stress factor (Barcelo *et al*., 1988a, b; Rascio *et al.*, 1993; Maksymiec *et al*., 1995; Skorzynska-Polit & Baszynski, 1997; Krupa & Moniak, 1998; Drążkiewicz *et al*., 2003). Studies on the age-dependent response of maize plants to Cd stress have shown that the metal distribution in leaf segments depends on their age (Drążkiewicz *et al*., 2003). In mature leaf tissues, the chloroplast ultrastructure was damaged under Cd treatment, whereas it was unaltered in young tissues (Rascio *et al*., 1993). Also, Cd reduced the photochemical processes more in older than in younger leaf segments, but the functional status during the dark phase of photosynthesis was more impaired in the younger leaves (Drążkiewicz *et al.*, 2003).

The determination of the structural changes induced by cadmium is a crucial step to understand the metabolic processes targeted by this xenobiotic agent and may be helpful to designate relevant approaches maintaining the vital functions of plants. The main objective of the present study was to characterize the structural changes in tomato shoot tissues of different maturity and address their potential physiological implications in response to Cd toxicity.

MATERIALS AND METHODS

Plant growth

Tomato (*Solanum lycopersicum* L. var. Ibiza F1) seeds were germinated on a wet filter paper in a thermostat-controlled darkened chamber (70% relative humidity and 25°C). Six-day-old seedlings having similar size and weight were transferred to plastic beakers (6 L capacity, seven plants per beaker) filled with nutrient solution containing 1 mM $MgSO_4$, 2.5 mM $Ca(NO_3)_2$, 1 mM KH_2PO_4, 2 mM KNO_3, 2 mM NH_4Cl, 50 μM EDTA-Fe-K, 30 μM H_3BO_3, 10 μM $MnSO_4$, 1 μM $ZnSO_4$, 1 μM $CuSO_4$, 30 μM $(NH_4)_6Mo_7O_{24}$ (Ouariti *et al.*, 1997). After an initial growth period of 12 days, Cd was added as $CdCl_2$ at different concentrations (0, 5, 10, 20, 50 and 100 μM). Seedlings were grown at a photosynthetic photon flux density of 150 μmol m^{-2} s^{-1} under a day/night regime of 16/8 hrs and 24/18°C since tomato develops well within this temperature range. The nutrient solution was continuously sparged with air and was replaced every 4 days. The pH of the medium was checked and adjusted daily to 5.5 ± 0.1. After 10 days of Cd-treatment, plants were harvested and divided into roots and shoots. The second and third internodes and leaves from the shoot bottom of each plant were sampled and used for all subsequent analyses. Internode length and diameter were measured with a digital caliper. Removed leaves were photocopied and the leaf area was determined with a leaf area meter (LI-COR area meter, model LI-3100, LI-COR, Inc., Lincoln, NE). It is worth-mentioning that the second internode and leaf (N2 and F2, respectively) were developed before Cd addition to the culture medium, while the third internode and leaf (N3 and F3, respectively) took place during Cd-treatment.

Cadmium content

The cadmium concentration in the roots and shoots was determined by atomic absorption spectrophotometry (Perkin Elmer 300) after wet digestion of 0.1 gr of dried material in 5 ml of a strong acid mixture (HNO_3/$HClO_4$, 3:1, v/v) (Van Assche *et al*., 1988).

Light microscopy

Samples were taken from the middle section of the second and third internodes and leaves from the bottom of control and cadmium-treated plants. Leaf segments of *ca.* 0.5 cm length were fixed for 3 hrs at 4°C with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 (Sabatini *et al*., 1963), dehydrated in a graded ethanol series and propylene oxide, and embedded in Spurr's epoxy resin. Semi-thin sections (0.5 to 1 μm thick) were cut on a Reichert-Jung ultracut-E ultramicrotome, stained with toluidine blue (equal volumes of 1% basic toluidine blue and 1% sodium tetraborate), and examined and photographed with a photomicroscope (Leica). Stem sections were obtained using a cryomicrotome (Leitz Kryomat 1700*),* pre-in-

cubated for 1 hr in sodium hypochloride in order to eliminate the cellular contents, and treated with 30% acetic acid. Then, they were stained with alune carmin and photographed. The micrographs were digitized with a scanner (HP Scanjet 3800, Hewlett Packard, USA) and analysed with the software (ImageJ, National Institutes of Health Bethesda, MD) to measure stem and leaf cross-sectional thickness. A minimum of three samples were examined per each Cd treatment.

Statistical analysis

Three separate experiments were performed; the first on to measure biomass and Cd content, the second one for determining internode length and diameter and leaf area, and the last one to realise microscopic and morphometric investigations. Two replicate beakers per treatment were used, with seven plants per beaker. The experiments were duplicated to ensure that the data obtained were reliable. Data presented are the mean values of 20 samples (for plant biomass) and 6 samples (for Cd content and morphometric assessments). For each parameter, data were subjected to a one-way ANOVA analysis. When the effect was significant ($p < 0.05$), differences between means were evaluated for significance by using Tukey's (HSD) test.

RESULTS

Effects of cadmium accumulation on plant growth

Cd accumulation (μg g^{-1} DW) was four-fold higher in roots than in shoots (Fig. 1). Yet, at similar internal Cd accumulation levels, shoot growth appeared to be more Cd-sensitive as compared to that of roots (Fig. 1). Indeed, the internal Cd concentration leading to a 50% reduction of biomass production was 240 μg g^{-1} DW and 2730 μg g^{-1} DW for shoots and roots, respe-

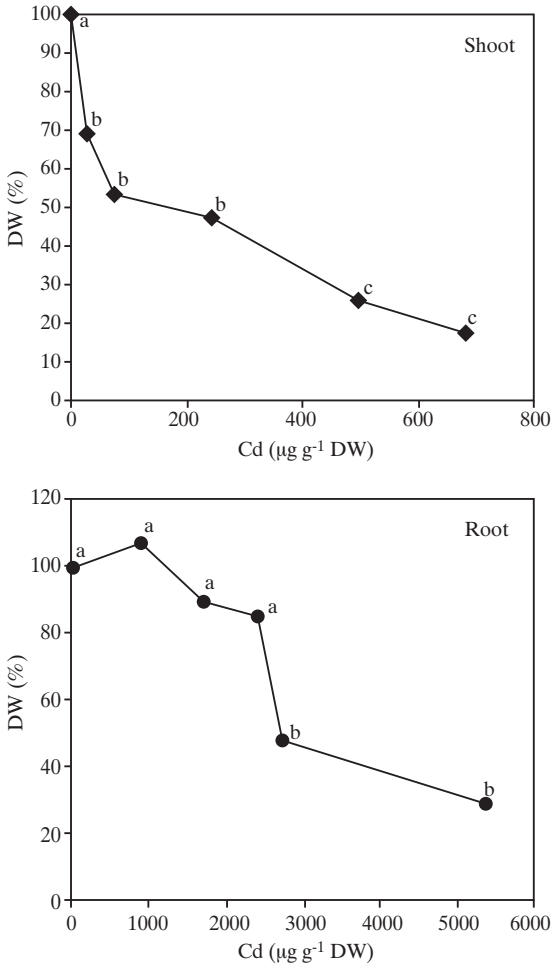

FIG. 1. Shoot and root dry mass (DW) variations (expressed in percent of control) as function of endogenous cadmium accumulated in plant organs after 10 days exposure to different metal doses in the culture medium. Values followed by the same letters are not significantly different (n = 20, $p < 0.05$) as resulted from Tuckey's test.

TABLE 1. Effect of different $CdCl_2$ concentrations on growth (mg DW) and dry weight/fresh weight (DW/FW) ratio of internodes and leaves after 10 days of treatment. Means (n = 20) followed by the same letters are not significantly different ($p < 0.05$) as resulted from Tuckey's test. Values as percentages of controls are given in parentheses. N2, second internode; N3, third internode; F2, second leaf; F3, third leaf

	N2		N3		F2		F3	
Cd (μM)	DW (mg)	DW/FW	DW (mg)	DW/FW	DW (mg)	DW/FW	DW (mg)	DW/FW
0	30.0^{a} (100)	0.036	45.2^{a} (100)	0.034	110.4^{a} (100)	0.081	173.7^{a} (100)	0.088
5	13.4^{b} (45)	0.036	17.0^{b} (38)	0.036	74.9^{b} (68)	0.084	119.3^{b} (69)	0.093
10	13.0^{b} (43)	0.037	11.1^{b} (24)	0.039	65.9^{b} (60)	0.082	91.4^{b} (53)	0.099
20	7.5^{b} (25)	0.041	6.9^{c} (15)	0.048	37.6^{b} (34)	0.088	46.1^{c} (26)	0.106
50	4.3^{c} (14)	0.059	2.7^{d} (6)	0.070	23.3^{c} (21)	0.101	16.7^{d} (10)	0.116
100	2.4^{c} (8)	0.092	1.1^{e} (2)	0.102	19.7^{c} (18)	0.111	7.1^{e} (4)	0.134

TABLE 2. Cadmium effects on internode length and diameter and leaf area after 10 days exposure to different $CdCl_2$ concentrations. Means ± confidence intervals (n = 6) followed by the same letters are not significantly different ($p < 0.05$) as resulted from Tuckey's test. Values as percentages of controls are given in parentheses. N2, second internode; N3, third internode; F2, second leaf; F3, third leaf

Cd (μM)	N2 Length (cm)	N3	N2	N3 Diameter (mm)	F2	F3 Leaf area (cm^2)
0	2.9 ± 0.7^a (100)	4.2 ± 0.6^a (100)	5.1 ± 0.6^a (100)	4.7 ± 0.5^a (100)	17.1 ± 2.1^a (100)	27.3 ± 2.0^b (100)
5	2.2 ± 0.5^a (76)	3.4 ± 0.5^a (81)	4.1 ± 0.5^a (81)	3.9 ± 0.3^a (83)	16.4 ± 1.6^a (96)	28.1 ± 1.5^b (103)
10	2.1 ± 0.5^a (72)	2.7 ± 0.7^b (64)	3.2 ± 0.3^b (63)	2.8 ± 0.3^b (59)	12.4 ± 0.8^b (72)	19.7 ± 1.1^c (72)
20	1.7 ± 0.7^a (59)	2.9 ± 0.5^b (69)	2.7 ± 0.3^b (53)	2.5 ± 0.1^b (53)	10.4 ± 0.8^c (61)	15.2 ± 1.0^d (56)
50	1.3 ± 0.5^b (45)	1.4 ± 0.4^c (33)	2.6 ± 0.2^b (51)	2.2 ± 0.1^c (47)	10.0 ± 0.8^c (58)	9.0 ± 0.7^e (33)
100	0.8 ± 0.3^b (27)	0.4 ± 0.1^d (9)	2.1 ± 0.3^b (41)	1.6 ± 0.2^d (34)	9.1 ± 0.6^c (53)	5.8 ± 0.4^f (21)

ctively (Fig. 1). A significant reduction of the internode growth was observed at 5 μM $CdCl_2$ (–55% and –62% of the control, respectively for N2 and N3) (Table 1). Fresh and dry weight of leaves (F2 and F3) were less affected than those of internodes (N2 and N3) in the presence of low Cd concentrations (5 and 10 μM $CdCl_2$), the leaf dry weight restriction not exceeding 47% of the control value (Table 1). At the highest Cd concentrations (50 and 100 μM $CdCl_2$), the growth of both N2 and F2 was generally less disturbed than that of N3 and F3 (Table 1). Cd-induced growth inhibition was concomitant with a gradual increase of the dry weight/fresh weight ratio in the studied internodes and leaves (Table 1). Stem length and diameter of both N2 and N3 were also negatively affected by Cd (Table 2), this effect being more acute in N3 than N2. Furthermore, leaf expansion was progressively suppressed after Cd-exposure (Table 2). Leaf area of F2 and F3 was close to that of the control at 5 μM $CdCl_2$, but a significant decline was observed at 10 μM $CdCl_2$, reaching up to 53% and 21% (for F2 and F3, respectively) of the control values in the seedlings challenged with 100 μM $CdCl_2$ (Table 2).

Effects of cadmium on stem anatomy

Light microscopy of transversal internode sections from the control plants (Fig. 2A) and Cd-treated plants at 100 μM $CdCl_2$ (Fig. 2B) clearly showed less ordered cell rows following Cd-exposure. Furthermore, the medullar and outer cortical layers showed misshapen cells with incorrect division plans, as compared to the corresponding control cells (Figs 2 and 3). A significant reduction of the tissue areas as well as of the number and size of the tracheary elements was observed in N2 and N3 treated with 100 μM CdCl2 (Table 3), these effects being more pronounced in N3 compared to N2 (Table 3). Transverse sec-

TABLE 3. Effects of cadmium (100 μM $CdCl_2$) on internode morphometric parameters (internode cross-sections) after 10 days exposure to Cd-treatment. Data are mean values of six measurements. Values (± confidence intervals) of the same line followed by the same letters are not significantly different ($p < 0.05$) as resulted from Tuckey's test. Values as percentages of controls are given in parentheses. N2, second internode; N3, third internode

Parameter	N2 0 μM Cd	100 μM Cd	N3 0 μM Cd	100 μM Cd
Internode layer thickness (μm)				
Epidermis	32 ± 1^a	25 ± 4^b (78)	28 ± 2^a	21 ± 3^c (75)
Cortical parenchyma	336 ± 28^a	210 ± 57^b (64)	178 ± 5^c	102 ± 12^d (57)
Medullar parenchyma	4264 ± 303^a	1629 ± 53^b (38)	4353 ± 167^a	1296 ± 23^c (30)
Metaxylem diameter	109 ± 33^a	62 ± 5^b (57)	88 ± 16^c	28 ± 1^d (34)
Protoxylem diameter	43 ± 8^a	26 ± 3^b (60)	35 ± 9^a	16 ± 4^c (46)
Number of xylem vessels/section	103 ± 7^a	65 ± 11^b (63)	109 ± 8^a	53 ± 15^d (49)

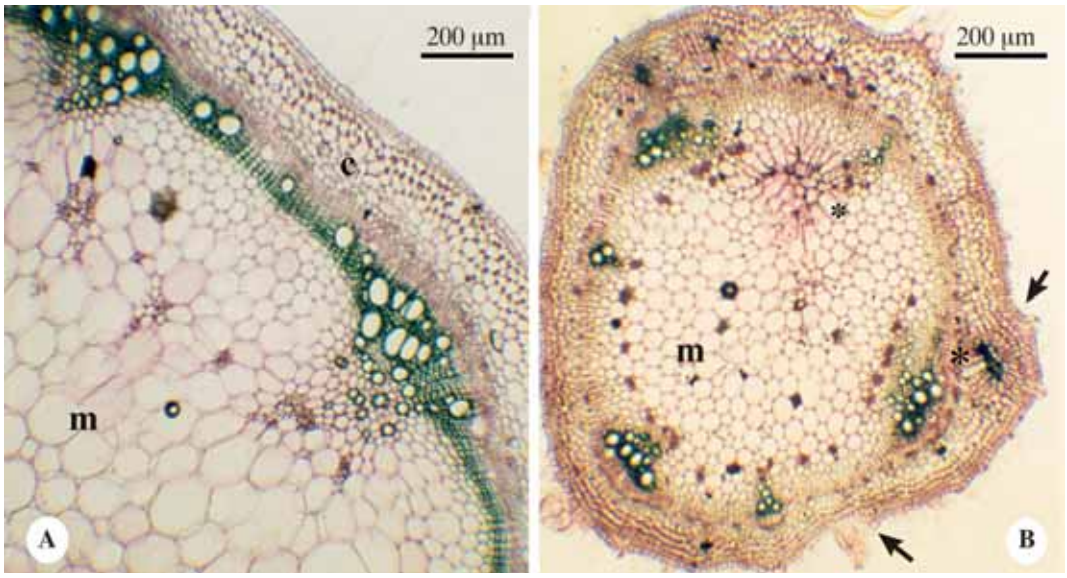

FIG. 2. Light micrographs showing second internode cross-sections of control (A) and 100 µM Cd-treated (B). Note the disturbance of the internode contour (arrowheads) and the presence of peculiar structures (asterisks) in the cortical (c) and medullar (m) parenchymas which exhibit a dense space bordered by rectangular-shaped cells.

tions obtained in N2 showed that the differences in tissue areas between the control (Fig. 2A) and the treated plants (Fig. 2B) were due to (i) a reduction of the cell size and (ii) a compressing of the cellular layers at certain places of the cortical parenchyma, involving a dissymmetry and a disturbance of the N2 contours, which exhibited a sinuous aspect (Fig. 2B, arrowheads). Yet, the number of cell layers constituting the cortical and the medullar parenchymas was poorly affected as compared to the control (Fig. 2).

N2 outline distortions observed under Cd stress (Fig. 2B, arrowheads) were accentuated by the presence of peculiar structures which occurred under the epidermis, and in the cortical or the medullar parenchymas. These structures consisted of a central cavity, bordered by rectangularly-shaped cells, flattened in the radial direction, and displaying thin walls (Fig. 3, asterisk). The cavity in the N2 tissues was generally formed, either by separation of parenchyma cells (schizogenously) (Fig. 4A) or by a disintegration of cells (lysigenously) (Fig. 4B).

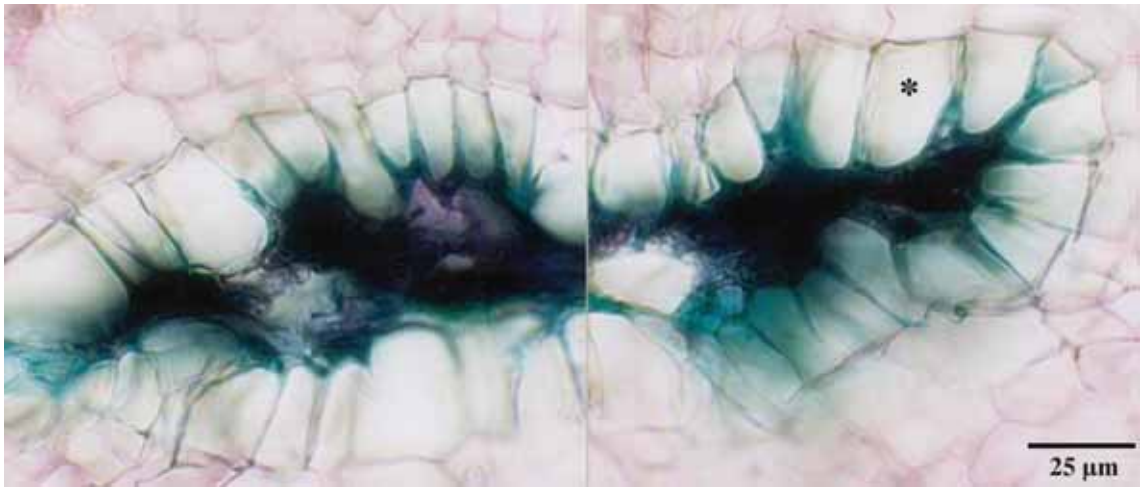

FIG. 3. Transverse sections of the second internode of plants treated with 100 µM $CdCl_2$ for 10 days. Note the presence of a peculiar structure formed by rectangularly-shaped cells (asterisk) which surround a cavity.

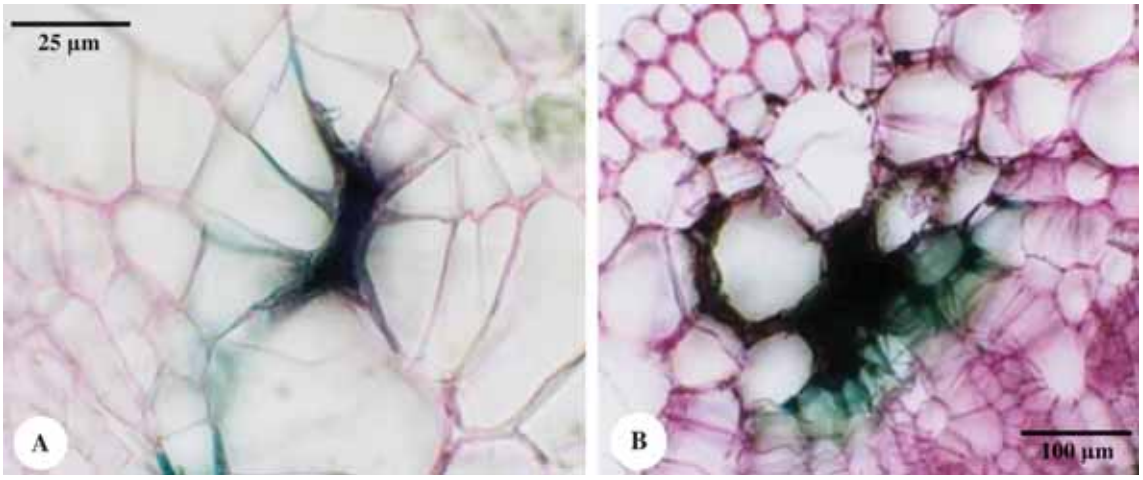

FIG. 4. Development of a peculiar structure at the cortical parenchyma of the second internode of 50 µM Cd-treated plants. A. Separation of the parenchyma cells to create a schizogenous cavity. B. A possible lysigenous mode of formation of a peculiar structure in the cortical parenchyma of the second internode. Note the disintegration of the cells in the space of the cavity.

TABLE 4. Morphometric assessments of leaves (cross sections) of plants grown in nutrient solutions containing 100 µM $CdCl_2$. Data are mean values of six measurements. Values (± confidence intervals) of the same line followed by the same letters are not significantly different ($p < 0.05$) as resulted from Tuckey's test. Values as percentages of controls are given in parentheses. F2, second leaf; F3, third leaf

	F2		F3	
Parameter	0 µM Cd	100 µM Cd	0 µM Cd	100 µM Cd
Leaf layer thickness (µm)				
Upper epidermis	33 ± 2^a	24 ± 1^b (73)	32 ± 3^a	19 ± 2^c (59)
Palisade parenchyma	131 ± 10^a	92 ± 4^b (70)	142 ± 18^a	84 ± 7^c (59)
Spongy parenchyma	198 ± 16^a	162 ± 6^b (82)	211 ± 19^a	148 ± 11^c (70)
Lower epidermis	25 ± 4^a	19 ± 2^b (76)	29 ± 5^a	15 ± 1^c (52)
Entire lamina	371 ± 31^a	301 ± 12^b (81)	416 ± 47^a	266 ± 21^c (64)

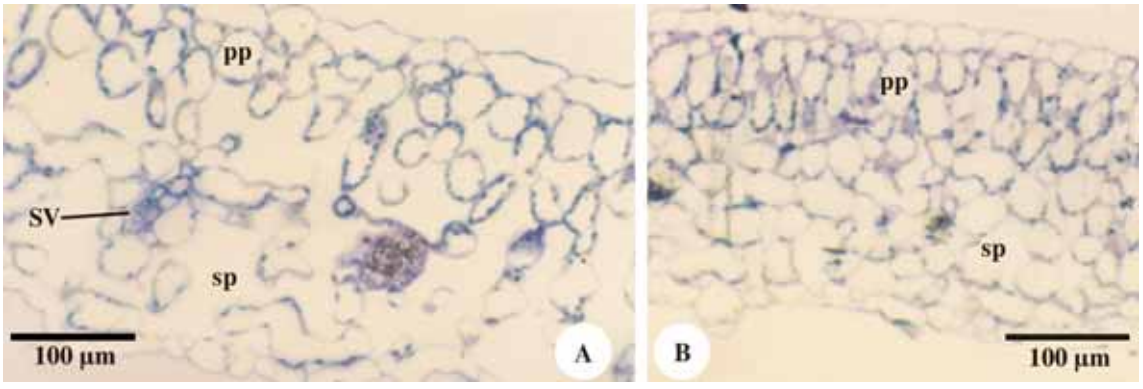

FIG. 5. Light micrographs showing transverse sections of control (A) and 100 µM Cd-treated (B) F2 stained with toluidine blue. Note the decrease in cell size and reduced intercellular spaces caused by Cd. pp, palisade parenchyma; sp, spongy parenchyma; sv, secondary vein.

Effects of cadmium on leaf anatomy

Anatomical observations on the second leaf (F2) revealed that despite the Cd-induced decrease in leaf blade thickness, the number of cell layers remained close to that of the control leaves, even at 100 μM $CdCl_2$ (Fig. 5A-B). The reduction of both cell size and intercellular space volume observed in F2 (Fig. 5B) clearly indicates that Cd altered leaf tissue expansion. Indeed, cadmium was shown to induce a pronounced restriction of the leaf tissues, much more significant in F3, than in F2 (Table 4). The thickness of the spongy parenchyma was particularly affected by high Cd-treatment. For instance, the thickness of palisade and spongy parenchymas in F2 of control plants was 131 μm and 198 μm, respectively. Cd-challenged plants showed thinner F2 leaves with significantly reduced palisade and spongy parenchymas thickness (92 μm and 162 μm, respectively). In F3 of Cd-treated plants, the thickness of palisade and spongy parenchymas was 84 μm and 148 μm, respectively.

Transverse sections revealed that leaf blade thinning was due to the reduced size of the mesophyll cells, probably related to the gradual increase of the leaf dry weight/fresh weight ratio in F2 and F3 (Table 1).

DISCUSSION

Excess cadmium triggers a wide range of biochemical effects and structural disturbances in plants, often accompanied by visual toxicity symptoms (Djebali *et al*., 2002, 2005, 2008; Zoghlami *et al*., 2006). Our results showed that increasing cadmium concentrations restricted the whole plant growth. Shoot growth was more sensitive to high Cd concentrations than root growth, although Cd accumulation was higher in the latter organ. At the highest concentrations (50 and 100 μM), root Cd contents represented respectively 84% and 88% of the total Cd incorporated in the plant. This is consistent with previous reports on tomato highlighting the extreme phytotoxicity of high tissue Cd concentrations (Djebali *et al*., 2002, 2005, 2008). The inhibition of seedling growth is a common effect of many heavy metals and is used as an indicator of Cd phytotoxicity (Ernst *et al*., 1992).

The decrease in shoot dry weight registered under Cd stress was concomitant with a significant reduction in both length and diameter of the internodes as well as in leaf area and thickness. The Cd-induced reduction of length and diameter of the internodes was tissue age-dependent. The reduction of internode diameter was associated with significantly lower cortical and medullar tissue extends, this effect being more pronounced in N3 than in N2. However, the number of cell layers constituting the stem (in cross-section) was unaffected by Cd-treatment. Restriction of stem tissue areas may be essentially attributed to the lower parenchyma cell size, and suggests a disturbance of the cellular expansion. The irregularity of cortical cell extension observed at highest Cd concentrations represents one of the aspects of this disturbance, leading to organ contour distortions. This phenomenon may also result from a disorder in the cell division process (Baryla *et al*., 2001). In our case, Cd interfered with the formation of regular cell rows and caused, in the most external layers, an enlargement of cells, which appeared the result of anomalous division planes. Simultaneously, Cd caused a significant reduction of the number and diameter of the xylem vessels. This response, commonly reported in other plant species like bean (Barcelo *et al*., 1988b), could involve a restriction of water flow translocation to shoots, and thus contribute to the perturbation of the plant water balance.

Cadmium that reached the leaves resulted in further physiological and structural damages. Leaf growth was inhibited and blade thickness was diminished owing to the reduced enlargement of mesophyll cells, resulting in increasing tissue dry weight/fresh weight ratio, especially in young (F3) leaves. Under Cd stress, stomatal conductance was found to sharply decline suggesting that stomatal functionality may be compromised (Perfus-Barbeoch *et al*., 2002). Changes in plant water relations with a decline in the transpiration rate have been observed in other Cd-exposed species (Costa & Morel, 1994; Baryla *et al*., 2001) and were ascribed to decreased leaf blade expansion (Haag-Kerwer *et al*., 1999). Although the reduction in leaf surface area is a rather common consequence of exposure to Cd, data regarding Cd effects on leaf cell enlargement are somewhat contradictory. In *Phaseolus vulgaris*, Cd caused a decrease in cell size (Barcelo *et al*., 1986), whereas in both *Brassica napus* and *Pisum sativum* it led to an increase of the mesophyll cell dimensions (Baryla *et al*., 2001; Sandalio *et al*., 2001). Exposure of submerged leaves to Cd in the aquatic plant *Elodea canadensis* was found to inhibit cell division but induced a significant enlargement of only one of the two cell layers constituting the leaf blade (Dalla Vecchia *et al*., 2005). Finally, in consistence with our findings, leaves of young wheat plants

became thinner and had less enlarged mesophyll cells following Cd-treatment (Kovačevic *et al*., 1999). This suggests that the changes in leaf cell enlargement caused by Cd may actually be due to specific morphogenetic effects rather than to impaired water balance.

The structure of foliar tissues provides another interpretation that could be used to explain their different Cd sensitivity. According to our findings, Cd led to a reduction of the mesophyll volume much more apparent in young than in old leaves (Table 4), whereas in Cd-treated *Pisum sativum*, leaf thickness and cell size increased (Sandalio *et al*., 2001). *Pisum sativum* further showed an impaired photosynthesis due to lower PSII activity (Chugh & Sawhney, 1999), in contrast to *Brassica juncea* (Haag-Kerwer *et al*., 1999) and *Arabidopsis thaliana* (Perfus-Barbeoch *et al*., 2002). It is well-known that leaves with high cell density have less efficient photosynthesis, and this also affects the intensity of gas exchanges. Niinemets *et al*. (1999) reported that leaf photosynthetic capacity scaled negatively with cell density. Furthermore, leaves with high cell density have a low intercellular space rate, which increases the resistance to gas diffusion into the tissues (Syvertsen *et al*., 1995). On the other hand, thick leaves have high photosynthetic potential (Niinemets *et al.*, 1999) and an ample lacunar tissue, which favours the diffusion of gases in the mesophyll. Cell density can provide further information about the importance of the apoplastic fraction and, as an indirect result, on the detoxifying potential (Lyons *et al*., 2000).

According to our findings, the intensity and the nature of Cd-induced structural modifications in tomato shoots differed according to the organ nature and age. Among the most spectacular effects generated by this pollutant at the stem level, was the appearance of "peculiar" formations, characterized by the presence of particular cells, delimiting a dense space, whose induction and localization remain tributary to the organ developmental stage and stress intensity. These formations were detected solely in N2 exposed to high cadmium treatment and could presumably play an active role in the response of the plant to this metal. Based on their mode of formation, aspects and localizations, one may hypothesize that they constitute exclusion sites where many compounds would be accumulated. To our knowledge, such structural modifications in response to Cd-stress have not been previously reported and require further investigation.

Despite numerous studies, the basic mechanisms through which Cd phytotoxicity takes place are still not completely understood, and remain an open question. This may be due to the fact that the apparent Cd effects may result from complex metal interferences with a great number of interacting physiological and metabolic processes, and also that in different species as well as in different organs a given activity may be differently and even oppositely affected by the metal. At the shoot level, Cd-induced structural disorders were more pronounced in the organs formed during treatment in comparison to those already present before stress application. These differences in Cd impact between internode and leaf types are likely related to the different stages of organ development at which they were first exposed to Cd. Taken together, our results point out that the Cd-induced structural deteriorations differ according to the nature and age of the affected organs, those built before the application of the stress being much less sensitive. This suggests the presence of response mechanisms and regulations typical for both the developmental stage and the nature of the contaminated tissue.

ACKNOWLEDGEMENTS

Thanks are extended to Dr. Ahmed Debez for his critical reading.

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