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Distribution and Phytotoxicity of Cadmium in Tomato Seedlings

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Thirty-day-old seedlings of tomato (*Lycopersicon esculentum* cv. Kwangsoo) were treated with various cadmium (Cd) concentrations (0, 10, 50, 100, and 500 μM) for up to 20 days, and the detailed distribution of absorbed Cd and its phytotoxicity in different plant parts (root, stem, and leaves) were investigated. The accumulation of Cd in plants increased with external Cd concentrations and Cd was strongly retained by roots, with less than 30% of the absorbed Cd being transported to shoots. Among the leaves, the lower positioned older leaves accumulated more Cd than the younger leaves. Furthermore, Cd-exposure not only reduced the dry weight and length of both shoot and root, chlorophyll levels in leaves, and levels of photosynthesis, but also enhanced the concentration of malondialdehyde (a lipid peroxidation product) in all plant parts. Our results indicate that the physiological impairment of tomato seedlings exposed to toxic levels of Cd may be related to the internal distribution of absorbed Cd, prolonged exposure, and oxidative stress in different plant parts.

Keywords: cadmium, distribution, lipid peroxidation, *Lycopersicon esculentum*

Heavy metal contamination of soils is one of the major environmental stresses, and cadmium (Cd) is one of the most toxic heavy metals in the present environment (Wagner, 1993). Cd is easily taken up by roots and translocated to different plant parts (Baker et al., 1994), and high accumulation generally causes growth inhibition and even plant death (Khan and Khan, 1983; Ouariti et al., 1997).

The high sensitivity of plants to heavy metals is thought to be due to inhibitory effects on enzyme activities (Krupa et al., 1993) and membrane transport (Keck, 1978), membrane damage (De Vos et al., 1991), reduced absorption of other cations (Khan and Khan, 1983), reduced transpiration (Costa and Morel, 1993) and photosynthesis (Clijsters and Van Assche, 1985), and chlorophyll destruction (Somashekaraiah et al., 1992). Both lipoxygenase-mediated lipid peroxidation and inhibition of antioxidant enzymes have been suggested to cause metal-induced phytotoxicity (Somashekaraiah et al., 1992).

Cadmium has been shown to enter roots by diffusion (Cutler and Rains, 1974) and root plasmalemma is the primary barrier to Cd^{2+} uptake (Tuner, 1973). Generally, Cd accumulation is higher in roots as compared to shoots (Salt et al., 1995a; Rauser and Meuwly, 1995) and absorbed Cd is mainly associated with cell walls (Hart et al., 1998) or sequestered in vacuoles (Li et al., 1997). However, the detailed distribution of Cd after uptake in various parts of plants is not known, and the basis for high shoot exclusion or restricted translocation to the shoot is poorly understood.

Tomato plants have been used as a research model to better understand metal uptake and metal-induced phytotoxicity (Wollgiehn and Neumann, 1995; Ouariti et al. 1997; Mazhoudi et al., 1997). In tomato seedlings grown in culture solution, the accumulation of Cd and Cu increased with external metal concentrations, and was considerably higher in roots than in shoots (Ouariti et al., 1997). However, the relationship between biomass and the distribution of Cd in various plant parts as well as the mechanism of phytotoxicity of such non-transition metal are not well understood.

In the present work, the distribution of Cd in root, stem and various leaves of tomato seedlings exposed to various Cd levels was investigated. In addition, the formation of malondialdehyde (MDA, one of the lipid peroxidation products induced by oxidative stress, Buege and Aust, 1978) in various tissues was investigated to determine whether Cd distribution is related to oxidative stress and subsequent growth inhibition. The information related to the translocation process from root to shoot will be valuable in developing safe food plants and in increasing crop plant yields. In plants where grains or fruits are the consumed tissues, the lower top/high root Cd accumulation would be desirable. Development of crops having the potential for lower leaf Cd accumulation where leaves are the consumed tissue would also be valuable. Furthermore, plants with high Cd accumulation might be used as efficient phytoremediation tools for metal-contaminated soil (Salt et al., 1995b).

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MATERIALS AND METHODS

Plant Material

Tomato seeds (*Lycopersicon esculentum* Mill. cv. Kwangsoo) were germinated and cultivated in pots containing a perlite:vermiculite (1:1) mixture in a controlled environment chamber at 25°C with 12 h of light (250 µM m^{-2} s^{-1}) and 70-80% humidity. Seedlings were supplemented daily with water and twice a week with modified Hoagland solution containing the following nutrients: 28.7 mg/L $NH_4H_2PO_4$, 0.71 mg/L H_3BO_3, 164.1 mg/L $Ca(NO_3)_2$, 0.02 mg/L $CuSO_4$, 2.66 mg/L ferric tartrate, 60 19 mg/l $MgSO_4$, 0.45 mg/L $MnCl_2$, 0.004 mg/L MoO_3, 151.65 mg/L KNO_3, and 0.055 mg/L $ZnSO_4$. Thirty days after germination, Cd was added daily to the pots as 0, 10, 50, 100, and 500 µM solutions of $CdCl_2$ in water. Leaves, stems, shoots and roots were collected from 20 plants undergoing each treatment after 10 or 20 d of Cd treatment; ten plants were dried for 48 h at 70°C and weighed for biomass and Cd determination, and the rest were taken for MDA and chlorophyll measurement. The experiments were conducted on at least five separate occasions, and mean values and SE were calculated.

Measurement of Cd

Leaves and stems were washed twice in deionized water, and the roots of intact plants were washed with ice-cold 5 mM $CaCl_2$ solution for 10 min to displace extracellular Cd (Rauser, 1987). The plant material was dried for 48 h at 70°C, weighed and ground into fine powder before wet ashing in $HClO_4$:HNO_3 (3:1) solution. Cd was determined directly by atomic absorption spectrophotometry (Varian 200AA equipped with SIPS, Australia) using an air-acetylene flame and Cd hollow-cathode lamp. To determine soil Cd concentrations, soils were dried and weighed, and washed for 24 h in sodium acetate buffer solution (Rauser, 1987). After filtration through filter paper (Whatman No. 1), Cd concentration was determined, following a similar method to that described above.

Measurement of Chlorophyll Level and Photosynthesis

Leaves collected at day 10 or 20 after Cd treatment were weighed and ground in 80% acetone. The resulting suspension was centrifuged for 10 min at 5,000 rpm. The chlorophyll content of supernatants was estimated according to the method of Arnon (1949): chlorophyll (µg) = $(20.2\times A_{645}) + (8.02\times A_{663})$.

The rate of net photosynthesis was measured in the growth chamber under ambient growth conditions with the portable photosynthesis system CI-500 (CID Inc USA). Relative CO_2 assimilation rates compared to control were calculated and are shown in Figure 3.

Measurement of Lipid Peroxidation

The level of lipid peroxides in the leaves and roots was determined as malondialdehyde (MDA) content by the thiobarbituric acid (TBA) reaction as described by Dhindsa et al. (1987). Fresh samples (100-500 mg) were weighed and ground in 5 mL of 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged at 10,000*g* at 4°C for 10 min. To a 1 mL aliquot of the supernatant, 4 mL of 20% trichloroactic acid containing 0.5% (w/v) TBA was added. The mixture was heated at 95°C for 30 min and then cooled on ice. The mixture was centrifuged at 10,000*g* for 15 min and the absorbance was measured at 532 and 600 nm. MDA concentration was calculated by subtracting the A_{532} from the A_{600} using a molar extinction coefficient of 155 mM $^{-1}cm^{-1}$.

RESULTS

Cd Accumulation and Distribution

In an effort to understand the time-course of accumulation and distribution of Cd in various tissues, seedlings were grown in a perlite:vermiculite (1:1) mixture supplemented daily with various Cd levels for up to 20 d. The seedlings accumulated substantial amounts of Cd in the leaves, stems and roots, and the accumulation in all tissues increased concurrently with the treatments applied (Table 1). Generally, Cd accumulation was highest in roots but was lowest in the younger or uppermost leaves on a dry weight basis (Cd µg g^{-1}dw). Following Cd uptake, roots accumulated 192.0 to 1502.9 µg g^{-1}dw after 10 d and 188.3 to 3,308.0 µg g^{-1}dw of the Cd after 20 d. Meanwhile, shoots accumulated 33.5 to 205.6 µg g^{-1} dw after 10 d and 41.8 to 341.6 µg g^{-1}dw of Cd after 20 d. Therefore, the Cd accumulation in shoots was approximately 10.5 to 28.8% of that in roots.

Although Cd content in tissues increased with exogenous Cd contents and prolonged treatment, a gradual decrease of the accumulation ratio (root-to-soil

Cd ratio) was also observed, indicating that the high Cd content in soil became a limiting factor for uptake. Another interesting aspect of the distribution data was that shoot-to-root Cd ratio decreased with increased Cd accumulation, indicating that high Cd content in the root became a limiting factor for the translocation of absorbed Cd. It is likely that the mobility of Cd from roots to shoot is limited at high Cd content or that leaves have limited capabilities of Cd accumulation at this growth stage. Although the 500 µM Cd treatment was 50 times more concentrated than the 10 µM Cd treatment, the concentration of Cd accumulated after 20 d in roots and shoots increased just 17.6- and 8-fold, respectively, which probably indicates an efficient Cd exclusion both at the root and from the shoot.

In shoots, Cd accumulation varied in the leaves. The first and second leaves contained more Cd than the third and fourth ones, and the highest Cd level was observed in the first leaves. Analysis of leaf Cd on a maturity basis showed that the older leaves (the first and second leaves), which occur in the lower part of seedlings, had more Cd than the younger leaves. Leaf-to-root Cd ratios were found to increase in older leaves. Since fifty day-old tomato plants were at physiologically immature, with emergence of a fourth leaf, any variation in Cd concentration in various tissues during this period should not have resulted from redistribution of Cd.

Although total Cd accumulation in stems increased with exposure levels, the stem-to-root Cd ratio was rather constant, indicating the increased Cd translocation from stem into leaves or the limited translocation of Cd from root into shoot.

Seedling Growth

The effects of Cd on seedling growth, expressed as dry weight and length of both shoot and root, are shown in Figure 1. Cadmium exposure induced the substantial decreases of dry weight and length of roots and shoots, depending upon the levels of Cd exposure and accumulation, duration of exposure time and tissues. In the shoot, early 10-d exposure increased dry weight slightly with 10 and 50 µM Cd but further exposure (20 d) decreased dry weight

Table 1. Distribution Cd in tomato seedings grown in perlite: vermiculite (1:1) mixture supplemented daily with various Cd concentrations for up to 20 d.

Cd treatment (µM)	Cd Content (µg g^{-1} dry wt.)										
	1st leaf[a]	2nd leaf	3rd leaf	4th leaf	Stem	Shoot[b]	Root	Soil	Stem/ Soil	Shoot/ Soil	Root/ Soil
10 d											
10	59.9±11.0[c]	56.8±4.6	35.4±6.3	NA[d]	30.4±9.8	33.5±1.3	192.0±27.6	3.5±0.1	8.7	9.6	54.6
	(33.9±5.2)[e]	(32.2±1.7)	(22.6±2.9)	NA	(17.3±6.1)	(18.9±0.2)					
50	97.51±1.1	91.31±2.4	67.2±17.3	NA	58.5±10.1	76.6±10.4	421.5±93.8	14.1±0.1	4.1	5.4	29.9
	(26.7±8.4)	(25.0±8.4)	(21.9±9.1)	NA	(15.4±6.5)	(21.0±7.0)					
100	113.4±2.3	113.8±19.4	85.9±2.9	NA	83.3±1.7	89.91±1.7)	542.2±54.1	51.5±0.2	1.6	1.7	10.5
	(20.9±3.3)	(20.8±6.8)	(16.2±1.7)	NA	(15.4±1.8)	(16.5±1.5)					
500	260.6±65.4	209.6±33.0	190.8±31.0	NA	281.0±54.9	205.6±52.1	1502.9±296.4	343.6±0.3	0.8	0.6	4.4
	(18.1±0.6)	(14.8±1.5)	(13.4±1.3)	NA	(19.7±1.2)	(15.3±7.5)					
20 d											
10	89.8±5.0	92.7±3.5	61.1±5.0	27.3±2.3	17.6±9.3	41.8±4.4	188.3±36.2	7.3±0.0	2.4	5.7	25.8
	(60.8±2.6)	(62.5±0.6)	(41.1±0.6)	(18.9±4.4)	(11.0±6.5)	(28.9±6.5)					
50	171.8±9.1	160.3±9.4	122.8±15.3	83.9±7.6	53.9±5.7	104.9±0.4	607.4±95.6	43.7±0.1	1.2	2.4	13.9
	(35.2±8.9)	(32.9±8.7)	(25.7±9.2)	(17.3±5.0)	(11.1±3.2)	(21.2±3.9)					
100	199.3±8.3	205.7±2.6	144.4±10.1	113.4±8.1	84.3±3.4	145.1±1.9	1092.8±100.0	54.1±0.1	1.6	2.7	20.2
	(22.3±4.3)	(22.9±4.0)	(16.3±3.9)	(13.0±4.3)	(9.4±1.7)	(16.1±2.1)					
500	408.6±45.0	390.4±9.0	306.8±23.3	257.1±3.0	456.7±45.0	341.6±15.4	3308.0±190.5	400.7±1.7	1.1	0.9	8.3
	(12.3±0.6)	(11.8±0.2)	(9.3±0.1)	(7.6±1.3)	(13.7±3.2)	(10.5±1.7)					

[a]Leaf number is from the bottom of the plant.
[b]Intact shoots containing leaves, stem and apex were used for analvsis of Cd content.
[c]Data are the means ±SE of five independent replicates.
[d]NA: data not available. The fourth leaves are not available at day 10.
[e]Relative accumulation ratio compared to root (%).

with over 50 μM Cd. No substantial length decrease was observed with up to 50 μM Cd during the early 10-d exposure, and longer exposure induced an increase of length with 10 μM Cd and a slight decrease with over 50 μM Cd levels. In the root, Cd exposure of over 50 μM resulted in a decrease of dry weight in shoots but no decrease of shoot length except with high Cd exposure (500 μM).

In terms of the amount of Cd accumulated (Table 1 and Fig. 1), after 20 d a final 41.8 μg g^{-1}dw of Cd accumulated in shoots exposed to 10 μM Cd, and induced an increase of shoot length but no change of dry weight. However, shoots exposed to 50 μM Cd for 20 d accumulated 104.9 μg, reducing both dry weight and length of shoot. Therefore, about 100 μg g^{-1}dw in the shoot might be required to reduce both dry weight and length of the shoot. In root exposed to 50 μM Cd, 421.5 and 607.4 μg of Cd accumulated after 10 and 20 d, respectively, resulting in reduced dry weight. However, the accumulation of up to 1,092.8 μg of Cd in the root was not enough to reduce root length even by day 20. Therefore, root growth was less sensitive to Cd accumulation than shoot growth in spite of the higher levels of Cd accumulation. The growth reduction observed at high doses of Cd closely coincided with considerable accumulation of this metal (Table 1), especially in the shoots.

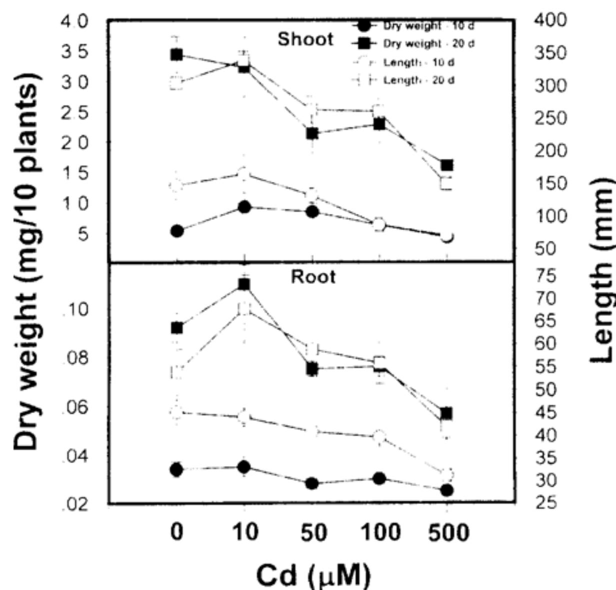

Figure 1. Dry weights and lengths of shoot and root of tomato seedlings exposed to various Cd levels for up to 20 days. Vertical bars indicate SE from five independent replicates.

Chlorophyll Formation and Photosynthesis

In spite of a substantial amount of Cd accumulation (Table 1), ten-day exposure to Cd was not enough to decrease chlorophyll contents in all the leaves studied (Fig. 2). However, further exposure to over 50 μM Cd for up to 20 days resulted in a substantial reduction in the chlorophyll contents of both the first and second leaves. Chlorosis was found in the older leaves, which might be related to preferential Cd accumulation in the leaves. In the third leaves, more accumulation (306.8 μg g^{-1} dw Cd content at day 20) was required to decrease chlorophyll content. These results imply that Cd-induced chlorophyll reduction depends upon Cd accumulation in tissues, leaf position, growth stage and exposure time.

Leaves grown at ambient CO_2 levels and subjected to Cd stress showed a reduction of photosynthesis (Fig. 3). Compared to the younger third leaves, more reduction was observed with the older first leaves. Therefore, Cd-induced reduction of photosynthesis

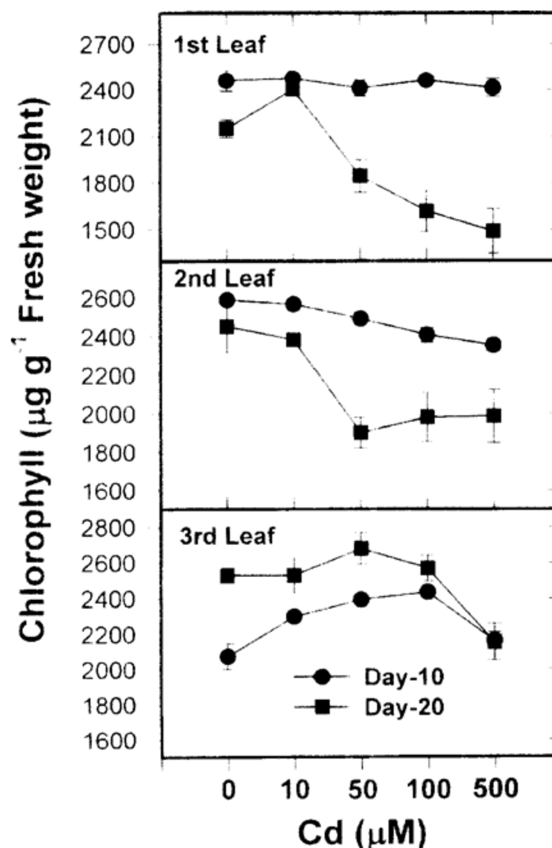

Figure 2. Chlorophyll levels of various leaves of tomato seedlings exposed to various Cd levels for up to 20 days. Vertical bars indicate SE from five independent replicates.

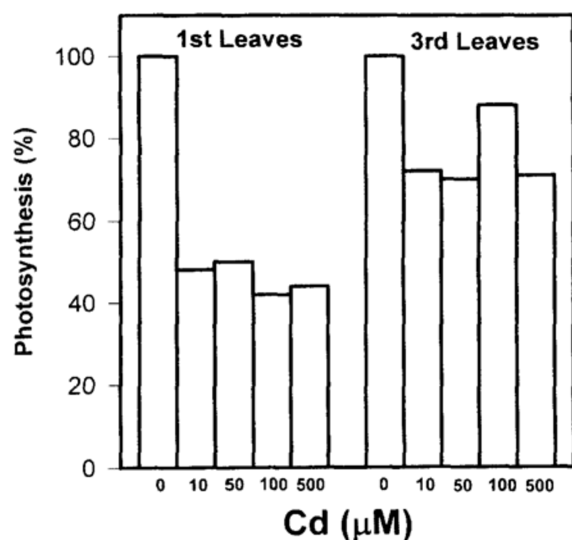

Figure 3. Photosynthesis of the first and the second leaves of tomato seedlings exposed to various Cd levels for up to 20 days. Relative photosynthesis rates compared to control are shown.

might depend on leaf age and Cd accumulation. No decrease of chlorophyll level (Fig. 2) but a reduction of photosynthesis at a lower level of Cd exposure (10 μM, Fig. 3) implies that the lowered chlorophyll formation is not the only reason for the reduced photosynthesis.

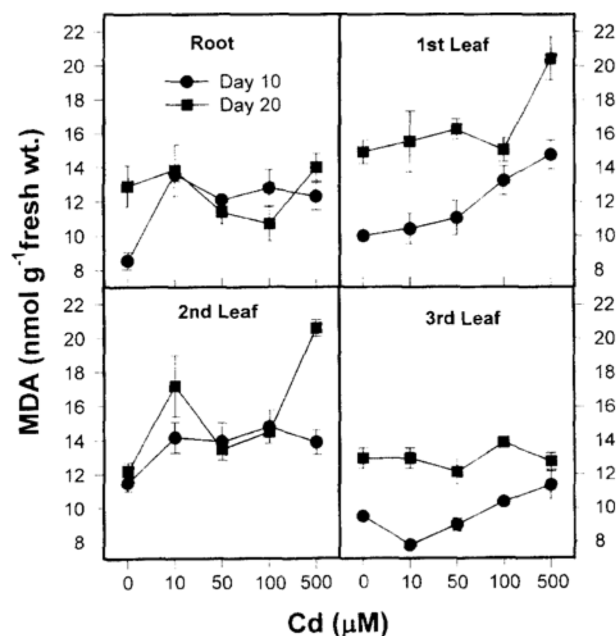

Figure 4. MDA contents of tomato seedlings exposed to various Cd levels for up to 20 days. Vertical bars indicate SE from five independent replicates.

Lipid Peroxidation

To determine whether Cd-induced reduction of seedling growth, chlorophyll level and photosynthesis resulted from oxidative stress-induced lipid peroxidation, MDA formation was investigated. MDA has mainly been identified as a product of lipid peroxidation (Buege and Aust, 1978). Figure 4 shows a consistent increase in MDA level parallel to increased Cd levels, particularly at 10 d. In both roots and the first leaves, a substantial increase of MDA was observed at day 10 but no increase except at 500 μM Cd was observed at day 20. In the second leaves, MDA increase was observed at days 10 and 20. However, in the third leaves, MDA increase was observed only at day 10 in leaves with greater than 100 μM Cd exposure. Therefore, the extent of MDA production responding to Cd exposure varied among tissues and depended upon leaf age and exposure time.

DISCUSSION

Although a number of studies demonstrated a generally reduced transfer between roots and shoots (Page et al., 1972; Weigel and Jager, 1980), details have not been provided with respect to time and concentration in specific tissues to allow for distribution in the growing plant. Since translocation requires the movement of Cd across the endodermis, membrane integrity to allow the symplastic movement might be important for the continuous Cd accumulation in shoots. It is possible that the absorption ability of shoot tissues depends upon the extent of damage to the cell membranes. This could also help to explain why the proportion of absorbed cadmium in tissues fell towards the higher cadmium concentrations in soil (Table 1). Reduced dry weight (Fig. 1) and high MDA production observed at day 10 (Fig. 4) might indicate the extent of cell damage.

However, since high Cd retention in roots might be due to cross-linking of Cd to carboxyl groups of the cell wall (Barcelo and Poschenrieder, 1990) and/or to an interaction with thiol residues of soluble proteins (Leita et al., 1993), and since Cd was found mostly in the cell wall and in soluble fractions (Lozano-Rodriguez et al., 1997), high Cd accumulation in roots even with substantial cell damage might be possible. The continuous increase in Cd accumulation at higher Cd accumulation (Table 1) and high MDA production measured at day 10 (Fig. 4) in roots could be explained on this basis. No increase of MDA mea-

sured at day 20 might indicate that the root tissues exposed to Cd for prolonged periods were too disrupted to produce further MDA (Ouariti et al., 1997). Anatomical characteristics of roots may play an important role in the low leaf/high root character (Wagner and Yeargan, 1986). A better understanding of these root transport processes should facilitate the production of plants with an increased ability to accumulate Cd within their shoots and roots.

In leaves, Cd accumulation may be driven by active transpiration (Hardiman and Jacoby, 1984) since more accumulation in the mature leaves (the first and second leaves) was observed. It has been suggested that the stem behaves as a cation exchange column resulting in a chromatographic distribution of metals towards the top of the plant, and the total amount of Cd absorbed by bean plants could be elevated by inducing higher transpiration rates (Hardiman and Jacoby, 1984).

How Cd increases lipid peroxidation is not clearly understood. Heavy metals are involved in many ways in the production of active oxygen species that induce peroxidation of membrane lipids (Halliwel and Gutteridge, 1984). Metals are known to alter membrane lipid metabolism (Somashekaraiah et al., 1992; De Vos et al., 1993; Ouariti et al., 1997), damage cell membranes and cause them to leak (Wainwright and Woolhouse, 1975; Strange and Macnair, 1991). Since Cd is not an active transition metal as Cu is (De Vos et al., 1993), it does not directly generate damaging oxygen species (Ouariti et al., 1997). However, Cd and Cu enhance lipoxygenase activity (Somashekaraiah et al., 1992), and the products of the lipoxygenase reaction, mainly peroxy, alkoxy and hydroxyl radicals, are themselves reactive and can result in further membrane lipid deterioration leading to membrane permeability (De Vos et al., 1991).

Prominent Cd-binding complexers in plant cells are the Cd-inducible phytochelatins (Cys-rich peptides, Grill et al., 1985) with the structure $(\gamma\text{-Glu-Cys})_n$ (Meuwly et al., 1995). The biosynthetic pathway of these peptides involves GSH or its metabolites (Gupta and Goldsbrough, 1991), and GSH plays an important role in the control of oxidative stress in plant cells. Therefore, depletion of GSH due to synthesis of phytochelatins in the presence of heavy metals may result in an increase in oxidative stress (Strange and Macnair, 1991; De Vos et al., 1992; De Vos et al., 1993).

Cd-induced lipid peroxidation might be induced by reduced activities of antioxidant enzymes such as catalase, GSH-reductase and superoxide dismutase (Somashekaraiah et al., 1992; Cho, unpublished data) and subsequent low levels of these enzyme activities may result in the enhancement of free-radical-mediated lipid peroxidation (Foyer et al., 1994). The accumulation of MDA in tomato tissues (Fig. 4) could be explained on these bases, and Cd might be considered an oxidative-stress enhancing factor, although Cd is not a redox-active cation.

The reduction of chlorophyll content, particularly in mature leaves (Fig. 2) might be due to increased cell or tissue damage as estimated by MDA production (Fig. 3). However, in young leaves, lipid peroxidation might not be related to the chlorophyll formation, and photosynthesis or continuous production of new cells might replace the damaged cells. It has also been suggested that metals themselves inhibit chlorophyll synthesis (Clijsters and Van Assche, 1985) and interfere with photosystems (Siedlecka and Baszynski, 1993).

Our results shows that the presence of Cd in the tissues may be associated with rapid physiological damage as inferred from the reductions of dry weight (Fig. 1), chlorophyll content (Fig. 2) and photosynthesis (Fig. 3), and the differential distribution of Cd among the tissues of seedlings may explain the differences in sensitivity of tissues to this metal. Further, Cd-exposure enhanced MDA formation (Fig. 4) in all tissues studied, presumably due to Cd-induced oxidative stress. Therefore, physiological impairment of tomato seedlings exposed to Cd may be induced by several factors including the internal distribution of absorbed Cd, prolonged exposure and oxidative stress in different plant parts.

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