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Distribution and Phytotoxicity of Mercury in Tomato Seedlings Exposed to Mercury

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ABSTRCT: Thirty-day-old seedlings of tomato (*Lycopersicon esculentum*) were treated with different concentrations of $HgCl_2$ (0, 10 and 50 μM) for up to 20 days, and the detailed distribution of Hg absorbed and its toxicity in different plant parts (roots, stems and leaves) were investigated. The accumulation of Hg in plants increased with external Hg concentrations, and Hg is strongly retained by roots. Further, Hg content in leaves was various, showing more accumulation in older leaves. Seedlings exposed to toxic levels of Hg showed not only the reduction of dry weight and length of both shoot and root, and chlorophyll levels in leaves but also the enhancement of malondialdehyde (a lipid peroxidation product) formation in all plant parts investigated. These results suggest that physiological impairment of a plant exposed to Hg may be achieved by internal distribution of Hg absorbed and Hg-induced oxidative stress in different plant parts.

Key Words: Distribution, Lipid peroxidation, *Lycopersicon esculentum*, Mercury.

INTRODUCTION

Heavy metal contamination of soils is one of the major environmental stresses. Metals are 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 toxic effects of metals on plants are 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.* 1993), reduced absorption of other cations (Khan and Khan 1983), reduced transpiration (Costa and Morel 1993) and photosynthesis (Clysters and Van Assche 1985), and chlorophyll destruction (Luna *et al.* 1994). Active oxygen species(AOS) -induced lipid peroxidation has been suggested to cause metal-induced phytotoxicity (Somashekaraiah *et al.* 1992). However, the fundamental mechanism of metal toxicity has not yet been characterized, and little is known about the mechanisms related to absorption and phytotoxicity of Hg, a potent metal pollutant.

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

In the present work, the distribution of Hg in root, stem and leaves of tomato seedlings exposed to toxic levels of Hg (10 and 50 μM) 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 know whether Hg-induced toxicity is related to oxidative stress.

MATERIALS AND METHODS

Plant material

Twenty five seeds of tomato (*Lycopersicon esculentum* Mill. cvs. Kwangsoo and Seokwang) were germinated and cultivated in a pot (11 cm × 18 cm × 5 cm) containing perlite:vermiculite (1:1) mixture in a controlled environment chamber at 25℃ with 12 h of light (250 $\mu M\, m^{-2}\, s^{-1}$) 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, Hg was added daily to the pots as 0, 10 and 50 μM of $HgCl_2$ in water. Leaves, stems and roots were

collected from 20 plants of each treatment after 10 or 20 days of Hg treatment; ten plants were used to measure the lengths of stem and the primary root, dried for 48 h at 70℃ and weighed for biomass and Hg determination. The rest were taken for MDA and chlorophyll measurement. The experiments were conducted on at least three separate occasions, and mean values and standard errors (SE) were calculated.

Measurement of Mercury

Leaves, stems and roots were separated and washed in deionized water two times, and dried at 70℃ for 48 h. The dried tissues were weighed and ground into fine powder before wet ashing in $HClO_4$:HNO_3 (4:1) solution. Hg was determined directly by atomic absorption spectrophotometry (Verian 200AA equipped with vapor generative accessory, Australia) using a Hg hollow-cathode lamp without a flame.

Measurement of chlorophyll level

Collected leaves at day 10 or 20 after Hg treatment were weighed and ground in 80% acetone. The resulting suspension was centrifuged for 10 min at 5000 rpm and the chlorophyll content of supernatants was estimated according to Arnon (1949).

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 10000 g at 4℃ for 10 min. To 1 ml of the aliquot of the supernatant, 4 ml of 20% trichloroactic acid containing 0.5% (w/v) TBA were added. The mixture was heated at 95℃ for 30 min and then cooled on ice. The mixture was centrifuged at 10000 g for 15 min and the absorbance was measured at 532 nm and 600 nm. The concentration of MDA was calculated based on $A_{532} - A_{600}$ ($\epsilon = 155\ mM^{-1}cm^{-1}$).

RESULTS

Mercury accumulation and distribution

The seedlings accumulated substantial amounts of Hg in leaves, stems and roots, and the accumulation increased concurrently with increases of exogenous Hg levels (Table 1). Generally, Hg accumulation was highest in roots but was lowest in younger or uppermost leaves (the third leaves) on a dry weight basis (Hg μg/g dry wt). Following Hg uptake, roots accumulated maximal 1583.2 and 1213.7 μg/g dry wt) for 20 days in Kwangsoo and Seokwang, respectively. The Hg accumulation in shoots is approximately 1.8 to 7.8% and 2.2 to 4.8% of that in roots in Kwangsoo and Seokwang, respectively. Two cultivars responded to Hg treatment differently: Seokwang accumulated more Hg than Kwangsoo in response to 10 μM Hg whereas Kwangsoo accumulated more than Seokwang in response to 50 μM Hg.

Although Hg content in tissues increased with exogenous Hg levels, a gradual decrease of leaf-to-root Hg ratio was observed, indicating that high Hg content in roots became a limiting factor for translocation. It is likely that mobility of Hg from root to shoot is limited at high Hg content or that leaves have limited capabilities of Hg accumulation at this growth stage. Although 50 μM Hg treatment was 5 times higher than 10 μM Hg treatment, the concentration of Hg accumulated for 20 days in leaves increased just up to 1.1- and 1.6-fold in Kwangsoo and Seokwang, respectively, which probably indicates an efficient Hg exclusion from leaves.

In shoots, Hg accumulation was various among the leaves and the highest Hg level was observed in stems in both cultivars. Analysis of leaf Hg on maturity basis showed that the older leaves (the first and the second leaves), which occur in the lower part of seedlings, had more. Therefore, leaf-to-root Hg ratios were found to increase toward old leaves.

Since low accumulation (200 μg) with 10 μM Hg treatment in Kwangsoo showed higher shoot-to-root ratio (7.8%) whereas high accumulation (625.2 μg) with 10 μM Hg in Seokwang showed lower shoot-to-root ratio (2.2%), there might be a genotype-specific level of root accumulation for efficient translocation into shoot.

Seedling growth

Hg treatment induced the substantial decreases of dry weight and length of roots and shoots, depending upon the levels of Hg exposure and accumulation, duration of exposure time and kinds of tissue (Table 2). In shoot, early 10-days exposure to 10 μM Hg was enough to reduce dry weight and length in both cultivars. In roots, 10-days exposure to 10 μM Hg decreased both dry weight and length in Kwangsoo whereas the same level of exposure was not enough to decrease dry weight and length in Seokwang in spite of high Hg accumulation (625.2 μg, Table 1).

Table 1. Distribution of Hg in tomato seedlings grown in perlite:vermiculite (1:1) mixture supplemented with various Hg concentrations for 10 days. Data are the means±SE of at least three independent replicates.

Hg treatment (μM)	Hg content (μ gg^{-1} dry wt)						
	Cotyledon	1st leaf[a)]	2nd leaf	3rd leaf	Stem	Shoot	Root
				cv. Kwangsoo			
0	0	0	0	0	0	0	0
10	25.7±1.0 (12.9)[b)]	18.6±0.1 (9.3)	16.6±1.0 (8.3)	10.3±0.2 (5.2)	4.2±0.8 (2.1)	15.6±0.8 (7.8)	200.0±6.0
50	26.2±0.1 (1.7)	21.2±0.1 (1.3)	17.4±0.1 (1.1)	12.8±0.0 (0.8)	47.5±4.2 (3.0)	29.7±0.8 (1.8)	1583.2±43.0
				cv. Seokwang			
0	0	0	0	0	0	0	0
10	26.4±0.1 (4.2)	22.2±0.1 (3.6)	20.1±0.3 (3.2)	12.8±0.1 (2.0)	16.9±0.1 (2.7)	13.5±0.1 (2.2)	625.2±3.5
50	30.8±0.2 (2.5)	25.2±0.3 (2.1)	24.4±0.0 (2.0)	20.2±0.1 (1.7)	66.3±0.2 (5.5)	58.6±0.2 (4.8)	1213.7±10.7

[a)] Leaf number is from the bottom of the plant.
[b)] The numbers in parentheses indicate the relative accumulation ratio compared to root (%)

Table 2. Dry weight (g/10 plants) and length (mm) of shoots and roots of tomato seedlings grown in perlite:vermiculite (1:1) mixture supplemented with various Hg concentrations for up to 20 days. Data are the means±SE of at least three independent replicates.

Hg treatment (μM)	Shoot				Root			
	Dry weight		Length		Dry weight		Length	
	10-d	20-d	10-d	20-d	10-d	20-d	10-d	20-d
				cv. Kwangsoo				
0	0.766±0.035	3.317±0.153	171.7±2.1	274.2±2.0	0.028±0.002	0.064±0.005	51.2±2.0	54.3±1.5
10	0.566±0.035	2.767±0.104	156.8±1.0	250.0±2.2	0.024±0.002	0.050±0.003	38.0±1.0	50.0±2.6
50	0.296±0.027	0.833±0.029	142.7±1.5	203.2±3.6	0.015±0.004	0.044±0.002	30.2±1.9	37.5±0.5
				cv. Seokwang				
0	1.100±0.050	3.200±0.265	159.8±3.8	279.7±6.7	0.030±0.006	0.106±0.005	34.7±3.8	45.0±0.5
10	0.750±0.050	2.789±0.134	153.6±1.2	266.1±4.5	0.030±0.005	0.064±0.004	34.7±0.3	36.8±3.4
50	0.610±0.285	1.200±0.050	150.5±3.3	197.2±8.1	0.020±0.003	0.050±0.001	27.2±0.8	32.8±1.5

Chlorophyll formation

With a substantial amount of Hg accumulation (Table 1), ten-days exposure to 10 μM Hg was enough to decrease chlorophyll contents in the first and the second leaves (Table 2). However, in the younger third leaves, the same level of exposure did not decrease chlorophyll contents in Kwangsoo or decreased slightly in Seokwang. Hg treatment also altered the chlorophyll a/b ratio depending on cultivars and exposure period. Chlorophyll b content was higher in leaves treated with low Hg (10 μM), thus decreasing the a/b ratio compared with that of controls. However, the content was lower in leaves treated with high Hg (50 μM) for 10 days, thus increasing the a/b ratio particularly in Seokwang. Both the reduction of chlorophyll levels and the alteration of chlorophyll a/b ratio might indicate the impairment of photosynthetic apparatus.

Lipid peroxidation

Fig. 1 shows a consistent increase in MDA level paralleled to increased Hg levels, and indicates that a genotype-specific minimal level is required to increase MDA formation. In the first and the third leaves, MDA formation increased with 20-days exposure to 50 μ M Hg in Kwangsoo and both 10-days exposure to 50 μM Hg and 20-days exposure to 10 μM Hg in Seokwang. In the third leaves, MDA increase was observed at day-10 with 50 μ M Hg in both cultivars. However, in roots, a consistent increase in MDA level paralleled to Hg exposures in Kwangsoo and an abrupt increase at 10-days exposure to 50 μM Hg in Seokwang were observed.

DISCUSSION

Although a number of studies demonstrated a generally reduced transfer of metals between roots and shoots (Page *et al.* 1972, Weigel and Jager 1980), details have not been provided with respect to concentration in spefic tissues to allow for distribution in the growing plant. Since translocation will require the movement of Hg across the endodermis, membrane integrity to allow the symplastic movement might be important for the continuous Hg accumulation in both roots and shoots. Since the roots of cv. Seokwang had low MDA content but high Hg accumulation with 10 μM Hg treatment for 10 days, it is possible that the initial absorption ability of root tissues depends upon the extent of damage to the cell membranes and accumulation requires membrane integrity. However, since high metal retention in root might be due to cross-linking of metal to carboxyl groups of the cell wall (Barcelo and Poschenrieder 1990; Lozano-Rodriguez *et al.* 1997) and/or to an interaction with thiol residues of soluble proteins (Leita *et al.* 1993), high Hg accumulation in root even with a substantial cell damage might be possible. The high Hg accumulation (Table 1) with high MDA formation (Fig. 1) at 50 μ M Hg treatment in roots of Kwangsoo could be explained on this basis. Anatomical characteristics of roots may play an important role in the low leaf/ high root character (Wagner and Yeargan 1986).

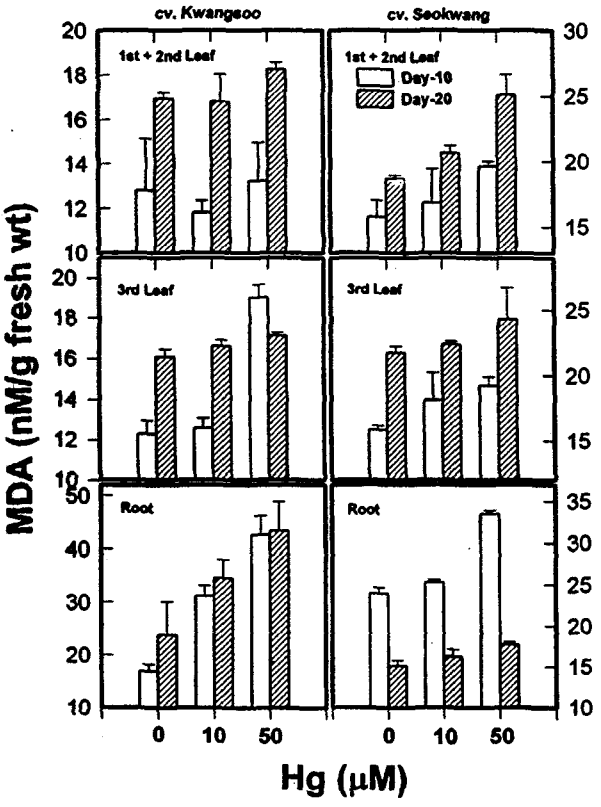

Fig. 1. Content of MDA in leaves and roots of tomato seedlings exposed to various levels of Hg for up to 20 days. Data are mean values of at least three independent experiments. SE are indicated by vertical bars.

In leaves, why older leaves positioned at lower part accumulated more Hg is not clear. Since 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 (Harman and Jacoby 1984), Hg accumulation may be driven by active transpiration and requires mature xylems.

Heavy metals are involved in many ways in the production of AOS such as O_2^-, OH^- and H_2O_2 that actively induce peroxidation of membrane lipids (Halliwel and Gutteridge 1984), and metals are known to alter the membrane lipid metabolism (Somashekaraiah *et al.* 1992, De Vos *et al.* 1993, Ouariti *et al.* 1997), damage cell membranes and make them leaky (Wainwright and Woolhouse 1975, Strange and Macnair 1991). Whether Hg is an active transition metal to generate AOS in plant tissues is not known. However, a non-active transition metal such as Cd enhanced 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). Since $HgCl_2$ enhanced lipid peroxidation in animal, as measured by the MDA formation and reduced glutathione (GSH) (Huang *et al.* 1996), the oxidative stress-induced lipid peroxidation might be one of the molecular mechanisms for cell injury in Hg toxicity. Our results showed that the determination of a MDA response may be used as a non-specific index of Hg-phytotoxicity which is more reliable than total content of Hg. Since chlorophyll degradation is a characteristic metabolic change of leaf senescence and it is usually accompanied or preceded by lipid peroxidation (Thomas 1986), the toxic effects may arise from the enhanced lipid peroxidation leading to injury to the chloroplast thylakoidal membranes (Reboredo 1997). Both reduction of chlorophyll levels and alteration of chlorophyll a/b ratio (Table 2) might indicate that chlo-

Table 3. Chlorophyll levels of tomato seedlings grown in perlite:vermiculite (1:1) mixture supplemented with various Hg concentrations for up to 20 days. Data are the means±SE of at least three independent replicates.

Hg treatment (μM)	Total chlorophyll content (μg g^{-1} fresh weight)			
	1st leaf + 2nd leaf[a]		3rd leaf	
	10-day	20-day	10-day	20-day
	cv. Kwangsoo			
0	2708±141 (2.70)[b]	2417±19 (2.62)	2618±5 (2.83)	2882±114 (2.68)
10	2472±44 (2.63)	2231±91 (2.61)	2651±74 (2.82)	2782±52 (2.60)
50	2339±46 (2.61)	2068±168 (2.60)	1992±157 (2.91)	2563±106 (2.62)
	cv. Seokwang			
0	2413±76 (2.79)	2233±41 (2.65)	2242±147 (2.79)	2767±23 (2.65)
10	2059±139 (2.75)	2116±139 (2.53)	2151±150 (2.75)	2534±7 (2.53)
50	2073±80 (2.82)	2154±73 (2.54)	1842±134 (2.82)	2491±4 (2.54)

[a] Leaf number is from the bottom of the plant.
[b] The numbers in parentheses indicate the chlorophyll a/b ratio.

roplasts are damaged and photosynthesis is impaired.

Based on the present work, it can be concluded that the accumulation of Hg in tomato seedlings increased with external Hg concentrations and Hg was strongly retained by roots, with less than 8% of the absorbed Hg being accumulated in shoots. Both the amount and the distribution of Hg determined in the tissues of a plant might be associated with physiological damage as inferred from reduction of dry weight (Table 2) and chlorophyll (Table 3). The toxic effects might be resulted from Hg-induced oxidative stress, as evidenced by the increase of lipid peroxidation (Fig. 1). MDA formation may be an efficient indication of initial cellular phytotoxicity induced by Hg.

The information related to the translocation process of metal from root to shoot will be valuable to develop safe food plants and yield increase of crop plants. Likewise, this knowledge could allow deeper insight into the molecular mechanisms of plant tolerance to metal-induced stress and the development of an efficient phytoremediation tool for metal-contaminated soil.

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