Journal of Plant Biology, March 1999, 42(1) : 41-48

Changes in Hydrogen Peroxide Content and Activities of Antioxidant Enzymes in Tomato Seedlings Exposed to Mercury

Un-Haing Cho* and Jung-O Park
Department of Biology, Changwon National University, Kyungsangnamdo 641-773, Korea

Thirty-day-old seedlings of tomato (*Lycopersicon esculentum* Mill.) were treated with various Hg concentrations (0, 10, and 50 μM) for up to 20 days, and the hypothesis that Hg induces oxidative stress leading to the reduction of biomass and chlorophyll content in leaves was examined. The accumulation of Hg in seedlings increased with external Hg concentration and exposure time, and Hg content in roots exposed to 50 μM Hg for 20 days was about 27-fold higher than that in shoots. Furthermore, Hg exposure not only reduced biomass and chlorophyll levels in leaves but also caused an overall increase of endogenous H_2O_2, lipid peroxidation products (malondialdehyde), and antioxidant emzymes activities such as superoxide dismutase, catalase, and peroxidase in leaves and roots. Our results suggest that the suppression of growth and the reduction of chlorophyll levels in tomato seedlings exposed to toxic Hg levels may be caused by an enhanced production of active oxygen species and subsequent high lipid peroxidation.

Keywords: mercury, H_2O_2, lipid peroxidation, antioxidant enzymes, *Lycopersicon esculentum*

The exposure of plants to toxic metal ions causes reduced plant growth or plant death, coincidental with the alteration of membrane permeability of cells leading to the leakage of ions (De Vos et al., 1993) and pigment destruction (Luna et al., 1994). However, in spite of the considerable literature on the subject, the fundamental mechanism of metal phytotoxicity has not yet been characterized and little is known about the mechanisms related to absorption and phytotoxicity of mercury (Hg), a cytotoxic metal pollutant.

Active oxygen species (AOS) such as O_2^-, OH^- and H_2O_2 are commonly generated under stress conditions (Halliwell and Gutteridge, 1984) and are strong oxidizing species that can rapidly attack all types of biomolecules (Asada, 1996), thus disrupting the normal metabolism of the cell. Generation of AOS, particularly H_2O_2, has been proposed as a part of the signaling cascade leading to protection from stresses (Dat et al., 1998). For the protection from oxidative stress, plant cells contain both oxygen-radical-detoxifying (antioxidant) enzymes such as catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD), and non-enzymatic antioxidants such as ascorbate, glutathione and α-tocopherol (Asada, 1996; del Rio et al., 1998). SOD, the first enzyme in the detoxifying process, catalyzes the dismutation of O_2^- to H_2O_2 and O_2 (Fridovich, 1986), CAT mediates the cleavage of H_2O_2 evolving O_2 (Scandalios, 1993), and POX reduces H_2O_2 to H_2O using several reductants available to the cells (Foyer et al., 1994). Altered activities of these antioxidant enzymes and antioxidants commonly have been reported in plants, and are used frequently as indicators of stress (Koricheva et al., 1997; Kang et al., 1998).

In parallel to metal-induced tissue damage or cell death, alterations of both antioxidant enzyme activities (Somashekaraiah et al., 1992) and antioxidant levels (Sinha et al., 1997) as well as enhancement of both lipid peroxidation (Ouariti et al., 1997) and phytochelatin synthesis (Gupta and Goldsbrough, 1991) have been observed, so metal-induced phytotoxicity may be mediated by oxidative stress. However, the changes in AOS metabolism and the enzyme activities involved in scavenging AOS in response to exposing plants to toxic metals have not been investigated in detail. In animals, $HgCl_2$ was found to enhance lipid peroxidation in several organs, as measured by the thiobarbituric acid reaction for malondialdehyde (MDA), and reduced glutathione level (Huang et al., 1996), indicating that the oxidative stress-induced lipid peroxidation may be one of the molecular mechanisms for cell injury in acute $HgCl_2$ poisoning. However, due to lack of data, it is difficult to assess the significance of oxidative stress induced by Hg in plants.

The objective of the present study was to investigate whether Hg-induced phytotoxicity expressed as growth inhibition and chlorophyll destruction in tomato seedlings is mediated by oxidative stress. We

*Corresponding author; fax +82-551-279-7440
e-mail uhcho@sarim.changwon.ac.kr

report that the seedlings exposed to toxic doses of mercury (up to 50 μM) produce H_2O_2 and the activities of related antioxidant enzymes are altered, indicating that Hg-induced phytotoxicity is mediated by oxidative stress.

MATERIALS AND METHODS

Plant Material

Seeds of tomato (*Lycopersicon esculentum* Mill. cv. Seokwang) 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, Hg was added daily to the pots as 0, 10, and 50 μM solutions of $HgCl_2$ in water. Plants collected from each treatment after 10 or 20 d of Hg treatments were dried for 48 h at 70°C and weighed for biomass and Hg determination. For measurements of H_2O_2, MDA, chlorophyll and antioxidant enzyme activities, fresh samples were weighed and used. The experiments were conducted at least in triplicate at different times, and mean values and standard errors were calculated

Measurement of Hg

Leaves and roots were separated and washed in deionized water two times, and dried at 70°C for 48 h. The dried tissues were weighed and ground into a fine powder before wet ashing in $HClO_3$:HNO_3 (4:1, v/v) solution. Hg was determined with an atomic absorption spectrophotometer (Varian 200AA equipped with vapor generative accessory, Australia) using a Hg hollow-cathode lamp.

Measurement of Chlorophyll and Lipid Peroxidation

Leaves collected 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. The chlorophyll content of the supernatant was estimated according to the method of Arnon (1949).

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).

Measurement of H_2O_2

Content of H_2O_2 in plant tissues was determined based on the modified method of Patterson et al. (1984). Fresh leaves or roots (150-300 mg) were frozen in liquid nitrogen and ground to a powder in a mortar together with frozen 5% TCA (1.5 mL) and activated charcoal (45 mg). The homogenate was centrifuged at 18,000g for 10 min at 0°C. The supernatant was filtered through a nylon filter (45 μm, MSI) and the filtrate was adjusted to pH 8.4 with 17 M ammonia solution. After re-filtration through a nylon filter, a 500 μl aliquot was brought up to 1 ml by adding 500 μl of colorimetric reagent. The colorimetric reagent was made daily by mixing 1:1 (v/v) 0.6 mM 4-(2-pyridylazo)resorcinol (disodium salt) (Sigma) and 2% titanium (IV) chloride (diluted from 20% $TiCl_4$ in conc. HCl and adjusted to pH 8.4) (Kanto Chemical Co. Inc., Japan) and was maintained in ice until use. The mixture was incubated for 60 min at 45°C and contents of H_2O_2 ($\varepsilon = 3.67$ μM $^{-1}$cm $^{-1}$) were determined from A_{415} using a series of H_2O_2 solutions, 5-50 μM, as standards (30% H_2O_2, Sigma).

Measurement of Antioxidant Enzymes

All samples were prepared for enzyme analyses by homogenizing the fresh tissue material with a mortar and pestle and a small amount of sand in a solution (5 mL/1 g fresh weight) containing 50 mM KH_2PO_4/K_2HPO_4 (pH 7.0), 10g L^{-1} PVP, 0.2 mM EDTA and 10 ml L^{-1} Triton X-100. After the homogenate was centrifuged at 12,000g for 20 min at 4°C, the supernatant was used for immediate determination of enzyme activities.

All spectrophotometric analyses were conducted with a Uvikon 922 spectrophotometer (Kontron Instruments, Italy). Activity of CAT was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 2 mL of 29.8 mM H_2O_2 in K-phosphate buffer (pH 7.0) and 1 mL extract (Beers and Sizer, 1952). Activity of SOD was assayed by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the modified method of Becana et al. (1986). The reaction medium comprised 0.25 ml 50 mM Na-phosphate buffer (pH 7.8)

with 0.1 mM Na_2EDTA, 2.73 mL $O^{\cdot}_2$-generating solution and 20.45 µL extract. The $O^{\cdot}_2$ generating solution contained 2.2 µM riboflavin, 14.3 mM methionine, and 82.5 µM NBT. Glass cells containing the mixture were placed in a cylindrical bath lined with aluminium foil at 25°C and fitted with a 22W fluorescent lamp. The reaction was initiated by turning the light on and the reduction of NBT was followed by reading the A_{560} for 10 min. Blanks were run the same way but without illumination. One unit of SOD was defined as the amount of enzyme which produced a 50% inhibition of NBT reduction under the assay conditions. Activity of guaiacol peroxidase (GPX) was measured by monitoring the H_2O_2-dependent oxidation of guaiacol at 470 nm (Chance and Maehly, 1955). One unit was defined as the enzymic amount which oxidizes 1 µM guaiacol min^{-1} (ε=26.6 $mM^{-1}cm^{-1}$).

Enzyme activity was expressed as units per g fresh weight. Contents of H_2O_2 and MDA were expressed on a g fresh weight basis.

RESULTS

Hg Accumulation, Seedling Growth and Chlorophyll Content

The content of Hg in tissues of tomato seedlings increased concurrently with the increase in external Hg level and exposure time (Table 1). Hg was more accumulated in roots than in upper plant parts; Hg contents in roots after 20 days was about 27-fold higher than that in shoots. The maximum accumulation of Hg was 1418.9 µg g^{-1} dry weight in roots with 50 µM Hg treatment for 20 days. Among the leaves, the first and the second leaves contained more Hg than the third ones, and the highest Hg level was observed in the first leaves.

The effects of Hg on seedling growth, expressed as dry weight and length of shoots and roots, are shown in Figure 1. Hg induced a substantial depression of both root and shoot dry weights, and this effect varied with the time of the exposure and the concentration of the exogenous Hg. The growth reduction observed at the high doses of Hg appeared to coincide with an increased accumulation of the metal (Table 1). However, 10 µM Hg treatment for 10 days (625.2 µg g^{-1} dry weight of Hg accumulation) was not enough to suppress both the dry weight and length of roots, indicating more resistance of roots to initial Hg stress than that of shoots.

Chlorophyll content (Fig. 2) showed a progressive decline with increasing Hg concentration in tissues (Table 1). With a substantial amount of Hg accumulation (Table 1), ten-day exposure to Hg was enough to decrease chlorophyll contents particularly in the first and the second leaves. However, the younger third leaves were more resistant to chlorophyll destruction since a 10-day treatment with 10 µM Hg was not enough to decrease the chlorophyll level.

H_2O_2 Production and Lipid Peroxidation

The effects of Hg on H_2O_2 production are presented for leaves and roots (Fig. 3). H_2O_2 content in roots was much higher than in leaves. Subjecting tomato seedlings to up to 50 µM Hg for 10 days increased the level of H_2O_2 in comparison with control plants, and the effect of Hg on the H_2O_2 level measured at day 10 was much higher in leaves than in roots. However, after 20 days of exposure, H_2O_2 level then decreased in the first and second leaves but increased slightly in both the third leaves and in roots.

Table 1. Distribution of Hg in tomato seedlings grown in perlite: vermiculite (1:1) mixture supplemented daily with various $HgCl_2$ concentrations for up to 20 days.

Hg treatment (µM)	Hg content (µg g^{-1} dry wt.)				
	1st leaf[a]	2nd leaf	3rd leaf	Shoot	Root
10 days					
0	0	0	0	ND	0
10	22.2±0.1[b]	20.1±0.03	12.8±0.04	ND	625.2±2.50
50	25.2±0.1	24.4±0.00	20.2±0.01	ND	1213.7±7.59
20 days					
0	0	0	0	0	0
10	33.7±0.02	24.7±0.02	19.4±0.06	16.6±0.3	819.5±26.50
50	33.7±0.06	33.7±0.06	20.9±0.16	51.9±0.8	1418.9±23.67

[a]Leaf number is from the bottom of the plant.
[b]Data are the means±SE of three independent replicates.
[c]ND: not determined

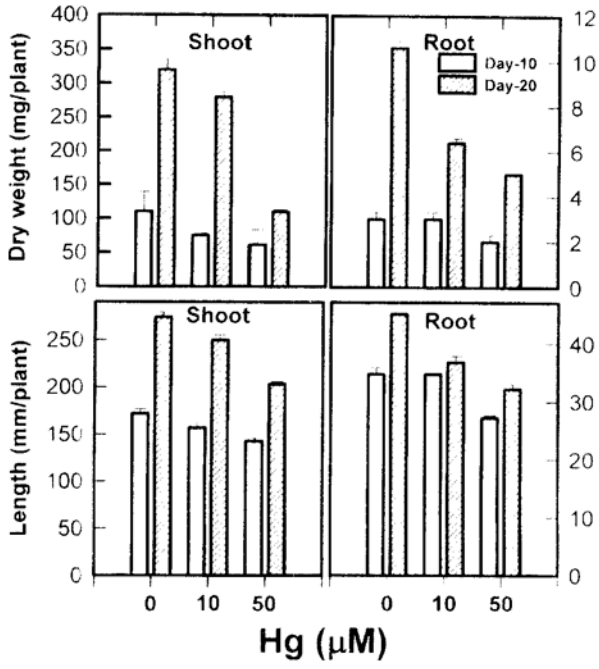

Figure 1. Dry weights and lengths of shoot and root of tomato seedlings exposed to various levels of Hg for up to 20 days. Data are mean values of at least three independent experiments. S.E. are indicated by vertical bars.

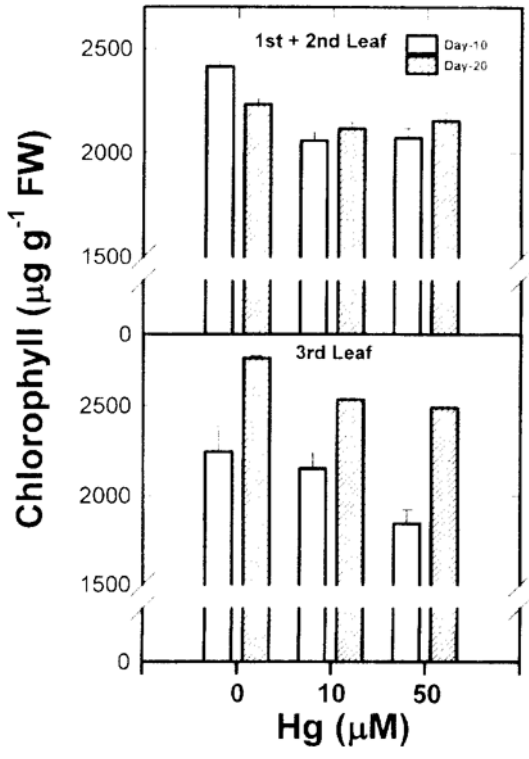

Figure 2. Chlorophyll levels of various leaves of tomato seedlings exposed to various levels of Hg for up to 20 days. Data are mean values of at least three independent experiments. S.E. are indicated by vertical bars.

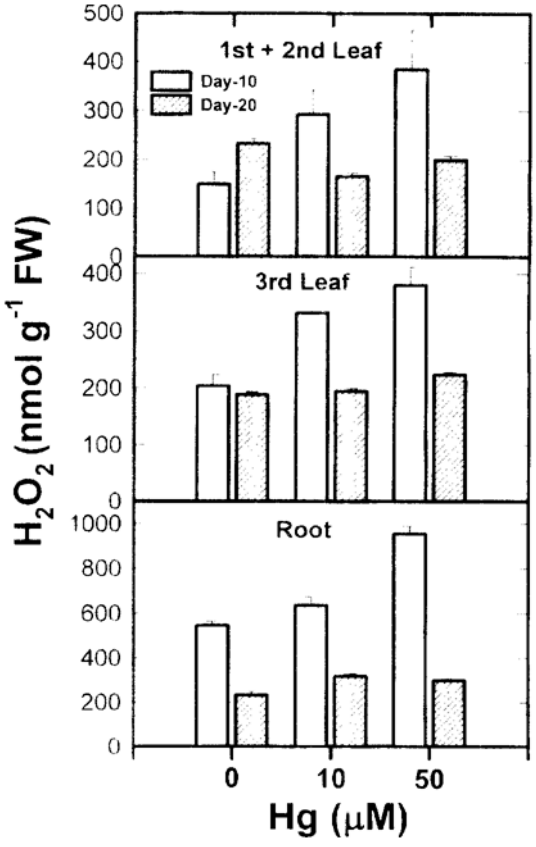

Figure 3. Content of hydrogen peroxide in leaves and roots of tomato seedlings exposed to various levels of Hg for up to 20 days. Data are mean values of at least three independent experiments. S.E. are indicated by vertical bars.

To determine if Hg-induced reduction of both seedling growth (Fig. 1) and chlorophyll level (Fig. 2) resulted from lipid peroxidation, MDA formation was investigated (Fig. 4). A consistent increase in MDA level paralleled both an increase of Hg accumulation and a decrease of chlorophyll level in leaves, and the MDA level observed at day 20 appeared to be related to the H_2O_2 level observed at day 10. In roots, the MDA level observed at day 20 was much lower than that at day 10 although Hg-exposure induced a substantial increase of MDA at all the levels of Hg treatment. Further, the pattern of MDA formation was in parallel with that of H_2O_2 formation in roots.

Antioxidant Enzymes

The activities of SOD, CAT and GPX were measured to ascertain whether Hg-exposure influenced these antioxidant enzymes (Figs. 5-7). All enzyme activities, estimated on a fresh-weight basis, were substantially increased by Hg-exposure, depending

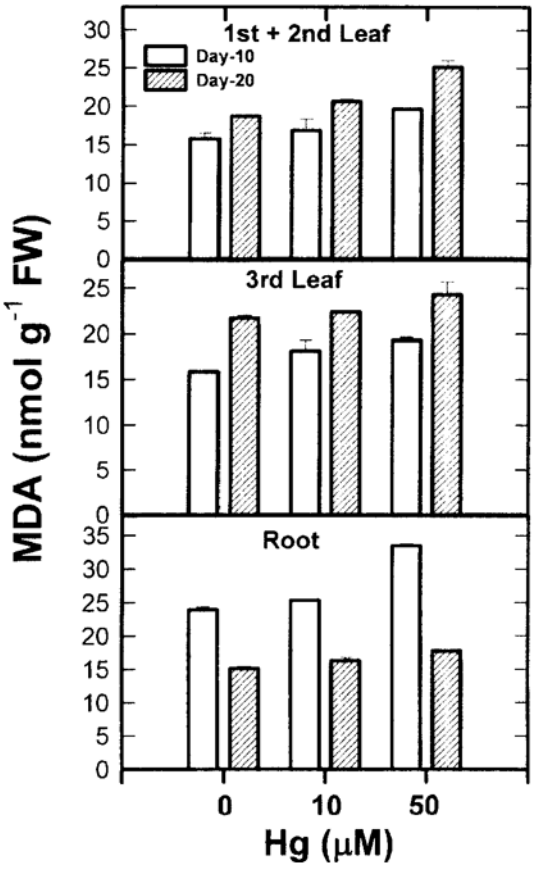

Figure 4. Content of MDA in leaves and roots of tomato seedlings exposed to various levels of Hg for up to 20 days. Data are mean values of at least three independent experiments. S.E. are indicated by vertical bars.

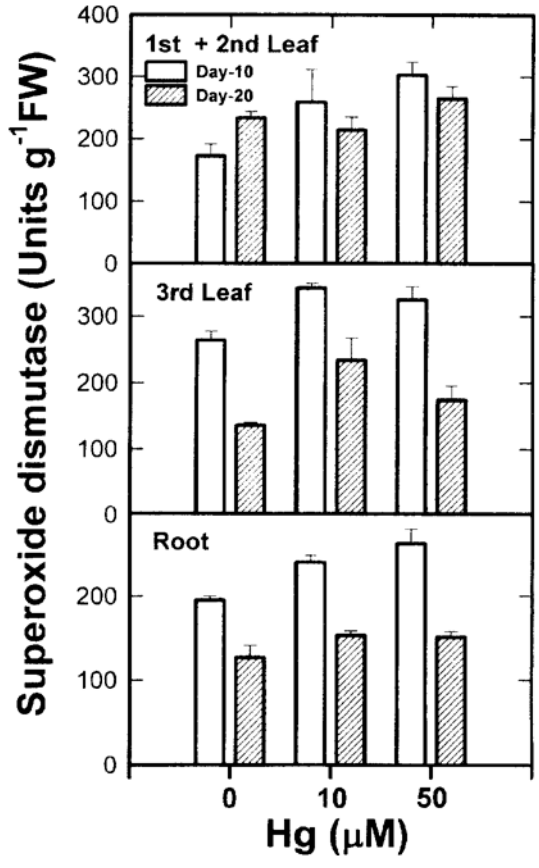

Figure 5. Activity of SOD in leaves and roots of tomato seedlings exposed to various levels of Hg for up to 20 days. Data are mean values of at least three independent experiments. S.E. are indicated by vertical bars.

on exposure time and treatment levels. Compared to the controls, the activity of SOD markedly increased in both leaves and roots exposed to Hg (Fig. 5). Ten-day exposure to 10 μM Hg was enough to increase the activity, and the increased SOD activities paralleled the levels of H_2O_2 formed in leaves and roots exposed to Hg for 10 days (Fig. 3). Examination of two enzymes which decompose the H_2O_2 generated by SOD indicated that the activities of CAT (Fig. 6) and GPX (Fig. 7) also increased in response to Hg exposure. The CAT activity in the first and the second leaves was not changed at day 10 with 10 μM Hg but increased at day 20 with 50 μM Hg compared to the controls (Fig. 6). In the third leaves, the CAT activity increased only with 50 μM Hg regardless of exposure time. Meanwhile, when subjected to Hg stress for up to 20 days, roots maintained higher levels of activity compared to the controls. The levels of H_2O_2 formed in response to Hg-exposure (Fig. 3) might be comparable to the activities of CAT particularly at day 20. The unexpected low H_2O_2 levels measured at day 20 (Fig. 3) with an increased SOD activity (Fig. 5) might be due to the increased CAT activity.

Mean GPX activity was higher in roots than in leaves (Fig. 7). In leaves, treatment with 50 μM Hg for 10 days or all treatments with Hg for 20 days resulted in a marked increase in GPX activity. In roots, all treatments with Hg for 10 days drastically reduced the enzyme activity but further treatments up to 20 days did not change (with 10 μM) or increased (with 50 μM) the activity. The results also indicated that the lowered GPX activity measured at day 10 in roots was recovered at day 20. The enhanced GPX activity might contribute to the reduction of H_2O_2 level measured at day 20 in leaves and roots.

DISCUSSION

Although a number of studies demonstrated that metals are generally immobilized to a far greater

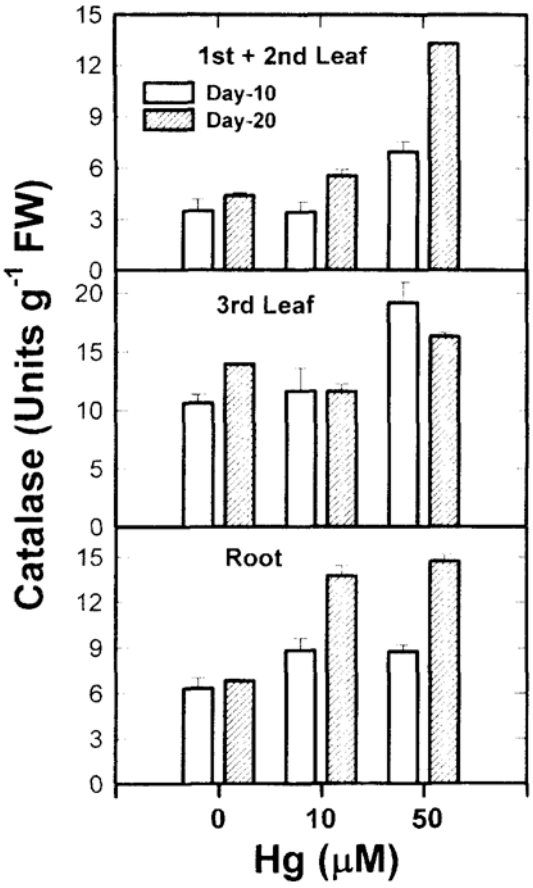

Figure 6. Activity of CAT in leaves and roots of tomato seedlings exposed to various levels of Hg for up to 20 days. Data are mean values of at least three independent experiments. S.E. are indicated by vertical bars.

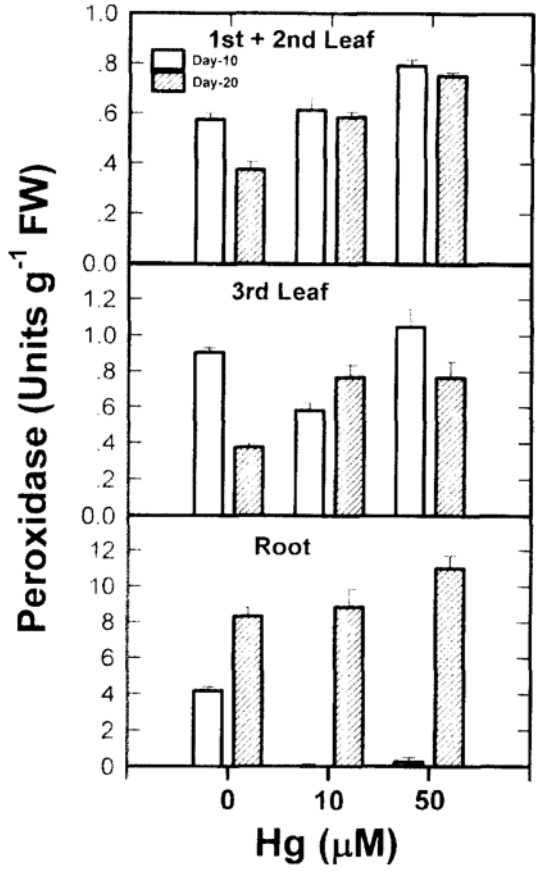

Figure 7. Activity of GPX in leaves and roots of tomato seedlings exposed to various levels of Hg for up to 20 days. Data are mean values of at least three independent experiments. S.E. are indicated by vertical bars.

extent at the site of metal uptake (Cataldo et al., 1981; Salt et al., 1995), 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 Hg across the endodermis, membrane integrity to allow the symplastic movement might be important for the continuous Hg accumulation in shoots. However, since metal accumulation is also found in the cell wall (Lozano-Rodriguez et al., 1997) or in apoplast (Neumann et al., 1997), high Hg accumulation in roots even with substantial cell damage might be possible. High Hg accumulation in roots (Table 1) in spite of high MDA production (Fig. 4) might be explained on this basis. The lowest accumulation in the third leaves implies that Hg movement through the xylem might be very slow.

The growth reduction observed at the various Hg levels (Fig. 1) closely coincided with the considerable accumulation of the metal, especially in the roots. The growth reduction might be due to both the reduction in chlorophyll contents in leaves (Fig. 2) and tissue damage indicated by enhanced lipid peroxidation (Fig. 4). It has also been suggested that heavy metals induce the deficiency in nutrients by reducing the uptake and transport of some mineral nutrients since metal accumulation in roots may block the entry or binding of ions to ion-carriers, such as Ca, Mg, P and Zn (Burzynski, 1987).

The reduction of chlorophyll content (Fig. 2) observed in this study might be due to increased cell or tissue damage, estimated by MDA production (Fig. 4). Destruction of lipid components of membranes by lipid peroxidation may cause membrane impairment and leakage. It has also been suggested that the reduction in chlorophyll content in the presence of metals is caused by an inhibition of chlorophyll biosynthesis (Van Assche and Clijsters, 1990).

The present study clearly indicates that Hg-exposure results in an increase in H_2O_2 content in plants

(Fig. 3). Although the mechanism of Hg-induced H_2O_2 formation is not known at present, heavy metals are known to be involved in many ways in the production of AOS (Halliwell and Gutterrifge, 1984). The H_2O_2 accumulation caused by Hg-exposure may occur in a manner similar to that in plants cold-stressed (Prasad et al., 1994). It is conceivable to suppose that a decrease of enzymic and non-enzymic free radical scavengers caused by heavy metals (De Vos et al., 1993) may also contribute to the shift in the balance of free-radical metabolism towards H_2O_2 accumulation, and H_2O_2 and O_2^- may interact in the presence of certain metal ions or metal chelates to produce highly reactive hydroxyl radicals (·OH). The increased H_2O_2 or ·OH production might be involved in the lipid peroxidation observed in tomato seedlings (Fig. 4).

The susceptibility to oxidative stress is a function of the overall balance between the factors that increase oxidant generation and those substances that exhibit antioxidant capability (Foyer et al., 1994). Some protective enzymes are activated in plants which stimulate production of oxygen free radicals, and an increase in SOD activity may be considered as circumstantial evidence of the enhanced production of AOS (Elstner et al., 1988). The enhanced SOD activity observed in this study (Fig. 5) might support the view that Hg-induced H_2O_2 formation (Fig. 3) results from oxygen free radicals including O_2^-.

The increased CAT activity (Fig. 6) might be related to the lowered H_2O_2 production observed at day 20 (Fig. 3), and indicates that the role of CAT might be critical to remove H_2O_2 induced by Hg. Although Cd (Somashekaraiah et al., 1992) inhibits CAT activity, the enzyme can take part in an efficient defense mechanism against Cu-induced oxidative stress in beans (Weckx and Clijsters, 1996).

Because of a significant increase in GPX activity and strong qualitative metal-specific changes in the GPX isozyme pattern (Van Assche et al., 1986; Mazhoudi et al., 1997; Chaoui et al., 1997), the role of GPX in the removal of H_2O_2 might be critical in metal-induced oxidative stress. GPX is a general POX which exists in the cytosol and cell wall and decomposes H_2O_2 (Asada, 1996). The activity of GPX was not changed in the first and the second leaves, was reduced in both the third leaves and roots with 10-day exposure, but was increased in all organs with 20-day exposure (Fig. 7). Therefore, GPX activity appeared to be expressed during long-term Hg exposure or after high Hg accumulation. It might be possible that Hg-induced GPX activity is associated with cell wall lignification and, consequently, with a decrease of root and stem growth (Fig. 1). POX has been postulated to stiffen the cell wall and POX-mediated lignification decreases the cell wall plasticity, and therefore reduces cell elongation which might represent a mechanical adaptation to stress conditions (Sanchez et al., 1995).

Based on the present work, it can be concluded that the amount of Hg in the tissues of tomato seedlings might be associated with the reduction of both biomass (Fig. 1) and chlorophyll contents (Fig. 2). Toxic concentrations of Hg cause oxidative stress, as evidenced by the increased H_2O_2 formation and lipid peroxidation in leaves and roots of seedlings. The reduction of both biomass and chlorophyll concentration (Fig. 2) might be resulted from lipid peroxidation-mediated cell damage in tissues. Hg-induced H_2O_2 formation may be associated with an increased activity of SOD for O_2^-. Although parallel increases in activities of CAT and POX occur and might contribute to lower H_2O_2 content, antioxidant potential in the tissues of seedlings might not be enough to block the lipid peroxidation process. The high POX activity might contribute to suppress the elongation of both shoots and roots. Summing up, we propose that the reduced growth of tomato seedlings exposed to toxic levels of Hg may be induced by the enhanced production of toxic oxygen species and subsequent lipid peroxidation.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of the Korean Research Foundation made in the program year of 1997.

Received January 13, 1999; accepted February 12, 1999.

LITERATURE CITED

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol **24**: 1-15

Asada K (1996) Radical production and scavenging in the chloroplasts. *In* AB Baker, ed, Photosynthesis and the Environment, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 123-150

Becana N, Aparcio-Tejo P, Irigoyen JJ, Sanchez-Diaz M (1986) Some enzymes of hydrogen peroxide metabolism in leaves and root nodules of *Medicago sativa*. Plant Physiol **82**: 1169-1171

Beers RF, Sizer IW (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem **195**:133-140

Burzynski M (1987) The influence of lead and calcium on the absorption and distribution of potassium, calcium, magnesium and iron in cucumber seedlings. Acta Physiol Plant **9**: 229-238

Cataldo DA, Garland TR, Wildung RE (1981) Cadmium distribution and chemical fate in soybean plants. Plant Physiol **68**: 835-839

Chance B, Maehly AC (1955) Assay of catalases and peroxidases. Methods Enzymol **2**: 764-775

Chaoui A, Mazhoudi S, Ghorbal MH, Ferjani EE (1997) Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.) Plant Science **127**: 139-147

Dat JF, Lopez-Delgado H, Foyer CH, Scott IM (1998) Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol **116**: 1351-1357

De Vos CHR, Ten Boukum WM, Vooijs R, Schat H, De Kok LJ (1993). Effect of copper on fatty acid composition and peroxidation of lipids in the roots of copper-tolerant and -sensitive *Silene cucubalus*. Plant Physiol Biochem **31**: 151-158

del Rio LA, Pastori GM, Palma JM, Sandalio LM, Sevilla F, Corpas FJ, Jimenez A, Lopez-Huertas E, Hernandez JA (1998) The activated oxygen role of peroxisomes in senescence. Plant Physiol **116**: 1195-1200

Dhindsa RS, Dhindsa P, Thorpe TA (1987) Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J Exp Bot **32**: 93-101

Elstner EF, Wagner GA, Schultz W (1988) Activated oxygen in green plants in relation to stress situations. Curr. Top Plant Biochem Physiol **7**: 159-187

Foyer CH, Lelandais M, Kunert KJ (1994) Photooxidative stress in plants. Physiol Plant **92**: 696-717

Fridovich I (1986) Biological effects of the superoxide radical. Arch Biochem Biophys **24**: 1-11

Gupta SC, Goldsbrough PB (1991) Phytochelatin accumulation and cadmium tolerance in selected tomato cell lines. Plant Physiol **97**: 306-312

Halliwell B, Gutteridge JMC (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J **219**: 1-14

Huang YL, Cheng SL, Lin TH (1996) Lipid peroxidation in rats administered with mercuric chloride. Biol Trace Elem Res **52**: 193-206

Kang KS, Lim CJ, Han TJ, Kim JC, Jin CD (1998) Activation of ascorbate-glutathione cycle in *Arabidopsis* leaves in response to aminotriazole. J Plant Biol **41**: 155-161

Koricheva J, Roy A, Vranjic JA, Haukioja E, Hughes PR, Hanninen O (1997) Antioxidant responses to simulated acid rain and heavy metal deposition in bitch seedlings. Environ Pollut **95**: 249-258

Lozano-Rodriguez E, Hernandez LE, Bonay P, Carpena-Ruiz RO (1997) Distribution of cadmium in shoot and root tissues of maize and pea plants. Physiological disturbances. J Exp Bot **306**: 123-128

Luna CM, Gonzalez CA, Trippi VS (1994). Oxidative damage caused by an excess of copper in oat leaves. Plant Cell Physiol **35**: 11-15

Mazhoudi S, Chaoui A, Ghorbal MH, Ferjani EE (1997). Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum*, Mill). Plant Science **127**: 129-137

Neumann D, Nieden UZ, Schwieger W, Leopold I, Lichtenberger O (1997) Heavy metal tolerance of Minuartia verna. J Plant Physiol **151**: 101-108

Ouariti O, Boussama N, Zarrouk M, Cherif A, Ghorbal MH (1997). Cadmium- and copper-induced changes in tomato membrane lipids. Phytochemistry **45**: 1343-1350

Patterson BD, McRae BA, Ferguson IB (1984) Estimation of hydrogen peroxide in plant extracts using titanium(IV). Anal Biochem **139**: 487-492

Prasad TK, Anderson MD, Martin BA, Stewart CR (1994) Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell **6**: 65-74

Salt DE, Prince RC, Pickering IJ, Raskin I (1995) Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol **109**: 1427-1433

Sanchez M, Revilla G, Zarra I (1995) Changes in peroxidase activity associated with cell walls during pine hypocotyl growth. Ann Bot **75**: 415-419

Scandalios JG (1993) Oxygen stress and superoxide dismutases. Plant Physiol **101**: 7-12

Sinha S, Gupta M, Chandra P (1997) Oxidative stress induced by iron in *Hydrilla verticillata* (l.f.) Royle: response of antioxidants. Ecotox and Environ Safety **38**: 286-291

Somashekaraiah BV, Padmaja K, Prasad ARK (1992) Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): involvement of lipid peroxides in chlorophyll degradation. Physiol Plant **85**: 85-89

Van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environ **13**: 195-206

Van Assche F, Put C, Clijsters H (1986) Heavy metals induce specific isozyme patterns of peroxidase in *Phaseolus vulgaris* L. Arch Int Physiol Biochim **94**: 60

Weckx J, Clijsters H (1996) Oxidative damage and defense mechanisms in primary leaves of *Phaselous vulgaris* as a result of root assimilation of toxic amounts of copper. Physiol Plant **96**: 506-512