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Mercury-induced oxidative stress in tomato seedlings

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Abstract

Mercury content and distribution as well as its effects on growth and oxidative stress were investigated in 30-day-old tomato seedlings (*Lycopersicon esculentum* Mill.). The content of Hg increased with external Hg concentrations, and was considerably higher in roots than in shoots. Among the leaves, the mature leaves accumulated more. Excess Hg suppressed biomass production of both roots and shoots and reduced chlorophyll content in leaves. Further, substantial increases of H_2O_2 content, malondialdehyde formation, and antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) were observed in Hg-stressed plants in comparison with controls. The results suggest that the phytotoxic effects of Hg in tomato seedlings may be achieved by an enhanced production of active oxygen species (AOS) and subsequent lipid peroxidation.

Keywords: *Lycopersicon esculentum*; Mercury; Oxidative stress; H_2O_2; Lipid peroxidation; Antioxidant enzymes

1. Introduction

The high toxicity of excessive metals has been known for a long time. The exposure of plants to metal ions causes growth inhibition or death of plants, coincidental with the alteration of membrane permeability of cells leading to the leakage of ions [1] and pigment destruction [2]. However, 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 $^1O_2$, O_2^-, $OH^\bullet$ and H_2O_2 are commonly generated under stress conditions [3] and are strong oxidizing species that can rapidly attack all types of biomolecules [4], thus disrupting the normal metabolism of the cell. Meanwhile, generation of AOS, particularly H_2O_2 has been proposed as part of the signaling cascade leading to protection from stresses [5]. For the protection from the 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 [4,6]. SOD, the first enzyme in the detoxifying process, catalyzes the dismutation of O_2^- to H_2O_2 and O_2 [7], CAT mediates the cleavage of H_2O_2 evolving O_2 [8], and POX reduces H_2O_2 to H_2O using several reductants available to the cells [9]. Altered activities of these antioxidant enzymes and antioxidants commonly have been reported in plants, and are used frequently as indicators of stress [10].

In parallel to metal-induced tissue damage or cell death, alteration of both antioxidant enzyme activities [11] and antioxidant levels [12] as well as enhancement of both lipid peroxidation [13] and phytochelatin synthesis [14] have been observed. Therefore, the metal-induced phytotoxicity may be mediated by oxidative stress. However, the changes in AOS metabolism and the enzymes activities involved in scavenging AOS in response of exposing plants to metal have not been investi-

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gated in detail. In animals, $HgCl_2$ enhanced lipid peroxidation in several organs, as measured by the thiobarbituric acid reaction for malondialdehyde (MDA), and reduced glutathione level [15], 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 present study is to investigate whether Hg-induced phytotoxicity expressed as growth inhibition and chlorophyll destruction in tomato seedlings is mediated by oxidative stress. The data show that tomato seedlings exposed to toxic dose of mercury (up to 50 μM) produce H_2O_2 and the activities of related antioxidant enzymes are altered, indicating that Hg-induced phytotoxicity can be mediated by oxidative stress.

2. Materials and methods

2.1. Plant material

Seeds of tomato (*Lycopersicon esculentum* Mill. cv. Seokwang) were germinated and cultivated in pots containing 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^{-1}, $NH_4H_2PO_4$, 0.71 mg l^{-1} H_3BO_3, 164.1 mg l^{-1} $Ca(NO_3)_2$, 0.02 mg l^{-1} $CuSO_4$, 2.66 mg l^{-1} ferric tartrate, 60.19 mg l^{-1} $MgSO_4$, 0.45 mg l^{-1} $MnCl_2$, 0.004 mg l^{-1} MoO_3, 151.65 mg l^{-1} KNO_3, and 0.055 mg l^{-1} $ZnSO_4$. Thirty days after germination, Hg was added daily to the pots as 0, 10 and 50 μM of $HgCl_2$ in water. Plants collected from each treatment after 10 or 20 days 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.

2.2. Measurement of Hg

Samples (leaves, shoots 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 $HClO_4$:HNO_3 (4:1, v/v) solution. Hg was determined by atomic absorption spectrophotometer equipped with vapor generative accessory (Verian 200AA, Australia).

2.3. 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 supernatant was estimated according to Arnon [16]. 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. [17]. The concentration of MDA was calculated based on $A_{532}-A_{600}$ ($\varepsilon = 155$ mM^{-1} cm^{-1}).

2.4. Measurement of H_2O_2

Content of H_2O_2 in plant tissues was determined based on the modified method of Patterson et al. [18]. 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 with activated charcoal (45 mg). The homogenate was centrifuged at 18 000 × g 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 NH_4OH. After re-filtration through a nylon filter, a 500-μl aliquot was brought 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, St. Louis, MO) and 2% titanium (IV) chloride (diluted from 20% $TiCl_2$ in 1 N HCl and adjusted to pH 8.4) (Kanto Chemical, 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_{508}, using H_2O_2 (30% Sigma) (5–50 μM) as a standard.

2.5. Measurement of antioxidant enzymes

All samples were prepared for enzyme analyses

by homogenization of the fresh tissue material with a mortar and pestle and a small amount of sand in a solution (5 ml g^{-1} fresh weight) containing 50 mM KH_2PO_4/K_2HPO_4 (pH 7.0), 10 g l^{-1} PVP, 0.2 mM EDTA and 10 ml l^{-1} Triton X-100. After the homogenate was centrifuged at 12 000 × *g* for 20 min at 4°C, the supernatant was used for immediate determination of enzyme activities. All spectrophotometric analyses were conducted on 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 29.8 mM H_2O_2 in KPO_4 buffer (pH 7.0) and 1 ml extract [19]. 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. [20]. 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_2^--generating solution and 20.45 μl extract. The O_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 22 W 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 reduced guaiacol at 470 nm [21]. One unit was defined as the enzymic amount which oxidizes 1 μM guaiacol min^{-1} ($\varepsilon = 26.6$ mM^{-1} cm^{-1}). Total activities (U) of enzymes and contents (nmol) of H_2O_2 and MDA were expressed on a fresh weight basis.

2.6. Statistical analysis

The results are the means ± S.E. of at least three independent replicates. The analyses of variance were computed on statistically significant differences determined based on the appropriate *F*-tests. The mean differences were compared utilizing Duncan's multiple range test.

3. Results

3.1. Hg accumulation and seedling growth

The content of Hg in tissues of tomato seedlings increased concurrently with increase in external Hg concentration and exposure time (Table 1). Hg was more accumulated in roots than in upper plant parts; Hg content 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 treated with 50 μM Hg for 20 days. Among the leaves, the mature first leaves contained the highest Hg level whereas the younger third leaves contained the least. The effects of Hg on seedling growth, expressed as dry weight and length of shoots and roots, are shown in Fig. 1. Hg-exposure induced a substantial depression of both root and shoot dry weights, and this effect varied as a 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 this metal (Table 1). However, 10 μM Hg treatment for 10 days (625.2 μg g^{-1} dry weight of Hg content) was not enough to suppress both the dry weight and length of roots, indicating less sensitivity of roots to initial Hg stress than that of shoots.

3.2. Chlorophyll levels, H_2O_2 production and lipid peroxidation

The effects of Hg on chlorophyll levels, H_2O_2 formation and lipid peroxidation are shown in Table 2. With a substantial amount of Hg accumulation (Table 1), Hg-exposure for 10 days was enough to decrease chlorophyll content particularly in the first and the second leaves. However, the younger third leaves were more resistant to chlorophyll destruction since 10-day treatment with 10 μM Hg was not enough to decrease chlorophyll level.

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 endogenous 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 roots.

To know whether lipid peroxidation was involved in the reduction of both seedling growth (Fig. 1) and chlorophyll level with Hg treatments, MDA formation was investigated. A consistent increase in MDA level paralleled both an increase of Hg accumulation and a decrease of chlorophyll

Table 1
Contents of Hg in tomato seedlings[a]

Hg treatment (μM)	Hg content (μg g^{-1} dry wt.)				
	1st leaf[b]	2nd leaf	3rd leaf	Shoot[c]	Root
Day-10					
0	0	0	0	ND[d]	0
10	22.2 ± 0.01[e] (3.5)[f]	20.1 ± 0.03 (3.2)	12.8 ± 0.04 (2.0)	ND	625.2 ± 2.50
50	25.2 ± 0.01 (2.1)	24.4 ± 0.00 (2.0)	20.2 ± 0.01 (1.7)	ND	1213.7 ± 7.59
Day-20					
0	0	0	0	0	0
10	33.7 ± 0.02 (4.1)	24.7 ± 0.02 (3.0)	19.4 ± 0.06 (2.4)	16.6 ± 0.3 (2.0)	819.5 ± 26.50
50	58.3 ± 0.01 (4.1)	33.7 ± 0.06 (2.4)	20.9 ± 0.16 (1.5)	51.9 ± 0.8 (3.7)	1418.9 ± 23.67

[a] Thirty-day-old seedlings were grown in perlite:vermiculite (1:1) mixture supplemented daily with different levels of $HgCl_2$ for up to 20 days, and Hg content was determined at day-10 and day-20 after treatment initiation.
[b] Leaf number is from the bottom of the plant.
[c] Intact shoots containing leaves, stem and apex were used for analysis of Hg content.
[d] ND, not determined.
[e] Values are means ± S.E. of at least three independent replicates.
[f] The numbers in parenthesis indicate the relative accumulation ratio compared to root (%).

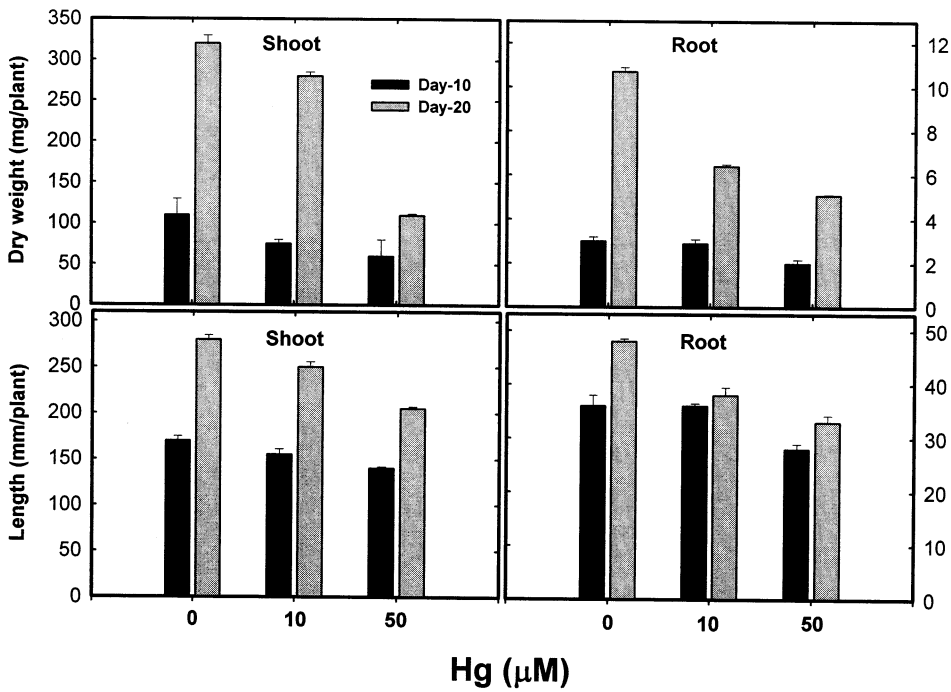

Fig. 1. Dry weights and lengths of shoot and root of tomato seedlings exposed to various levels of Hg for up to 20 days. Data are mean values of least three independent experiments. S.E. are indicated by vertical bars.

Table 2
Contents of chlorophyll, H_2O_2 and malondialdehyde (MDA) in various parts of tomato seedlings[a]

Hg treatment (μM)	Chlorophyll (μg g^{-1} FW)		H_2O_2 (nmol g^{-1} FW)			MDA (nmol g^{-1} FW)		
	1st+2nd leaf[b]	3rd leaf	1st+2nd leaf	3rd leaf	Root	1st+2nd leaf	3rd leaf	Root
Day-10								
0	2413.3 ± 24.0b	2243.0 ± 142.7b	149.6 ± 24.9a	203.8 ± 19.7a	545.6 ± 10.2a	15.8 ± 0.7a	15.8 ± 0.2a	23.9 ± 0.4a
10	2058.8 ± 40.3a	2151.2 ± 86.8b	292.8 ± 49.6b	331.5 ± 2.6b	635.9 ± 37.0b	16.8 ± 1.5a	18.1 ± 1.2b	25.3 ± 0.2b
50	2073.2 ± 46.2a	1842.3 ± 77.6a	384.6 ± 81.6c	379.7 ± 31.8c	955.5 ± 33.5c	19.6 ± 0.2b	19.3 ± 0.4b	33.5 ± 0.3c
Day-20								
0	2233.8 ± 10.1b	2766.6 ± 13.5b	233.3 ± 3.6c	188.8 ± 3.0a	232.2 ± 15.8a	18.7 ± 0.1a	21.7 ± 0.3a	15.1 ± 0.4a
10	2117.0 ± 30.6a	2534.6 ± 40.1a	165.7 ± 1.6a	193.9 ± 2.8b	317.4 ± 11.3b	20.6 ± 0.3b	22.4 ± 0.1b	16.2 ± 0.5ab
50	2154.2 ± 42.1a	2490.7 ± 10.9a	199.4 ± 10.6b	224.2 ± 1.7c	297.7 ± 7.8b	25.1 ± 0.9c	24.3 ± 1.4b	17.7 ± 0.2bc

[a] Thirty-day-old seedlings were grown and treated with $HgCl_2$ as described in Table 1, and the contents were determined at day-10 and day-20 after treatment initiation. Note: Values are means ± S.E. of at least three independent replicates. Values in a column followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.
[b] Leaf number is from the bottom of the plant.

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 treated. Further, the pattern of MDA formation paralleled that of H_2O_2 formation in roots.

3.3. Antioxidant enzymes

The activities of SOD, CAT and GPX were investigated to determine whether Hg-exposure influenced these antioxidant enzymes (Table 3). All enzyme activities, estimated on a fresh weight basis, were substantially increased by Hg-exposure, depending on exposure time and treatment levels. Compared to the controls, the activity of SOD markedly increased in both leaves and roots exposed to Hg. 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 (Table 2). Examination of two enzymes, which decompose the H_2O_2 generated by SOD, indicated that the activities of CAT and GPX 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. In the third leaves, the CAT activity increased 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 (Table 2) might be comparable to the activities of CAT particularly at day 20. The unexpected low H_2O_2 levels measured at day-20 with an increased SOD activity might be due to the increased CAT activity.

Mean GPX activity was higher in roots than in leaves. 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, and the

enhanced GPX activity might contribute to the reduction of H_2O_2 level measured at day-20 in leaves and roots.

4. Discussion

Although a number of studies have demonstrated that metals are generally immobilized to a far greater extent at the site of metal uptake [22,23], details have not been provided with respect to time and concentration in specific 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 shoots. However, since metal accumulation is also found in the cell wall [24] or in the apoplast [25], 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 (Table 2) indicates the extent of cell damage which might be explained on this basis. The lowest accumulation in the upper third leaves also implies that absorbed Hg is not readily mobilized and redistributed in the plants, and transpiration [26] may not be involved in Hg translocation.

The observed changes in the biomass of tomato seedlings were consistent with previous results obtained at high Hg in pea [27] and tobacco [28]. The growth reduction observed at the levels of Hg in treatments (Fig. 1) closely coincided with a considerable accumulation of this metal, especially in the roots. The growth reduction might be due to both the reduction in chlorophyll contents in leaves (Table 2) and membrane damage indicated as an enhanced lipid peroxidation (Table 2). 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 root may block the entry or binding of the ions such as Ca, Mg and K to ion-carriers [29].

Table 3
Activities of antioxidant enzymes in various parts of tomato seedlings[a]

Hg treatment (μM)	Enzyme activities (U g^{-1} FW)								
	SOD			CAT			GPX		
	1st+2nd leaf[b]	3rd leaf	Root	1st+2nd leaf	3rd leaf	Root	1st+2nd leaf	3rd leaf	Root
Day-10									
0	172.4 ± 19.3a	263.6 ± 14.0a	194.9 ± 5.1a	3.5 ± 0.7a	10.6 ± 0.8a	6.3 ± 0.7a	0.6 ± 0.0a	0.9 ± 0.0b	4.2 ± 0.2b
10	259.0 ± 53.0b	342.4 ± 7.9b	240.4 ± 8.2b	3.4 ± 0.6a	11.6 ± 2.0a	8.8 ± 0.8b	0.6 ± 0.1a	0.6 ± 0.1a	0.1 ± 0.1a
50	302.8 ± 21.7b	324.8 ± 20.4b	262.9 ± 17.7b	6.9 ± 0.6b	19.2 ± 1.8b	8.7 ± 0.4b	0.8 ± 0.1b	1.0 ± 0.0b	0.2 ± 0.2a
Day-20									
0	233.7 ± 10.1ab	135.5 ± 4.7a	126.3 ± 14.7a	4.4 ± 0.2a	14.0 ± 0.1ab	6.8 ± 0.1a	0.4 ± 0.0a	0.4 ± 0.0a	8.3 ± 0.3a
10	214.5 ± 20.8a	233.3 ± 34.1bc	152.8 ± 5.6b	5.5 ± 0.4b	11.6 ± 0.7a	13.7 ± 0.7b	0.6 ± 0.0b	0.8 ± 0.0b	8.8 ± 0.1a
50	264.5 ± 20.2b	173.4 ± 21.5b	150.7 ± 6.8b	13.3 ± 0.0c	16.3 ± 0.4bc	14.7 ± 0.5b	0.8 ± 0.0c	0.8 ± 0.1b	11.0 ± 0.4b

[a] Thirty-day-old seedlings were grown and treated with $HgCl_2$ as described in Table 1, and enzyme activities were determined at day-10 and day-20 after treatment initiation. Note: Values are means ± S.E. of at least three independent replicates. Values in a column followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.
[b] Leaf number is from the bottom of the plant.

The reduction of chlorophyll content (Table 2) observed in this study might be due to an increased cell or tissue damage estimated by MDA production (Table 2). Destruction of lipid components of membrane by lipid peroxidation causes membrane impairment and leakage. Meanwhile, it has also been suggested that the reduction in chlorophyll content in the presence of metal is caused by an inhibition of chlorophyll biosynthesis [30].

The present study clearly indicates that Hg-exposure results in an increase in H_2O_2 content in plants (Table 2). Although the mechanism of Hg-induced H_2O_2 formation is not presently known, heavy metals are known to be involved in many ways in production of AOS [2]. The H_2O_2 accumulation after Hg-exposure may be produced in a manner similar to H_2O_2 in plants cold-stressed [31]. It is conceivable that a decrease of enzymic and non-enzymic free radical scavengers caused by heavy metals [23] 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 the highly reactive hydroxyl radical ($^{\bullet}OH$). The increased H_2O_2 and $^{\bullet}OH$ production might be involved in the lipid peroxidation observed in tomato seedlings (Table 3). 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 [9,32]. Some protective enzymes are activated in plants when production of oxygen free radicals is stimulated by stresses, and increased SOD activity may be considered as circumstantial evidence for enhanced production of AOS [33]. The enhanced SOD activity observed in this study (Table 3) might support the hypothesis that the H_2O_2 resulted from oxygen free radicals including O_2^-.

The increased CAT activity (Table 3) might be related to the lowered H_2O_2 production observed at day 20 (Table 2), and indicated that the role of CAT might be critical to removal of H_2O_2 induced by Hg. Although Cd [11] inhibits CAT activity, the enzyme can take part in an efficient defense mechanism against Cu-induced oxidative stress in bean [34].

Because of a significant increase in GPX activity and strong qualitative metal-specific changes in the GPX isozyme pattern [35–37], the role of GPX in removal of H_2O_2 might be critical in metal-induced oxidative stress. GPX is a general POX which exists in both cytosol and cell wall and decomposes H_2O_2 [4]. The activity of GPX was not changed in the first and the second leaves, and was reduced in both the third leaves and roots with 10 days exposure, but was increased in all organs with 20 days exposure (Table 3). Therefore, GPX activity appeared to be expressed in a long-term Hg exposure or at 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 [38].

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 (Table 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 might result 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^- conversion. Although parallel increases in activities of CAT and POX occur and might contribute to lower H_2O_2 content, the 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 elongation of both shoots and roots. Summing up, it was proposed that the reduced growth of tomato seedlings exposed to toxic levels of Hg may be induced by an enhanced production of toxic oxygen species and subsequent lipid peroxidation.

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