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Effects of Mercury and Chromium on Peroxidase and IAA Oxidase Enzymes in the Seedlings of *Phaseolus vulgaris*

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Abstract: Phaseolus seedlings (*Phaseolus vulgaris* L.) in light conditions were treated with 2 different concentrations of 2 heavy metals, mercury (0.05 mM and 0.4 mM) and chromium (0.5 and 1.0 mM). Mercury was given in the form of $HgCl_2$ and chromium was given in the form of $K_2Cr_2O_7$. Peroxidase activity was measured with 4 different hydrogen donors (ferulic acid, caffeic acid, pyrocatechol and pyrogallol). Both mercury and chromium inhibited root and hypocotyl length. The inhibition was about 50%-80%, depending on the concentration of the heavy metal. Peroxidase and IAA oxidase activities showed a clear inverse relation with growth. Seedlings treated with a lower concentration of mercury had similar activities to those treated with higher concentrations of chromium. The role of peroxidase and IAA oxidase activities in defense mechanisms in response to heavy metal toxicity is discussed.

Key Words: chromium, mercury, metal toxicity, peroxidase activity, defense mechanism

Introduction

Peroxidases are of immense importance in a variety of cellular functions such as lignification, suberization, cell elongation, growth, regulation of cell wall biosynthesis and plasticity (1-3). Peroxidases are commonly classified as cytoplasmic or wall bound (ionically or covalently). In some instances, disease-specific isoperoxidases have been seen to appear in infected tissues (4) and these individual isozymes of peroxidase may have unique functions.

Heavy metals are known to cause irreversible damage to a number of vital metabolic constituents and important biomolecules. They also cause irreversible injury to the plant cell wall and cell membrane. Trace elements are necessary for the normal metabolic functions of the plant, but at higher concentrations these metals are toxic and may severely interfere with physiological and biochemical functions. The toxic threshold level of the metal in the tissue is defined by the 'stress point' for metal toxicity, and beyond this level the physiological state of the cell will be irreversibly damaged (5). This change is reflected by an increase in the activity of certain enzymes, and peroxidase induction is a general response of higher plants to uptake of toxic amounts of metals (6).

Mercury (Hg) is a highly toxic, nonessential, persistent, immutable and non-biodegradable metal that undergoes many changes during transfer through different trophic levels of the food chain. Hg occurs naturally in the environment and exists in several forms such as metallic mercury, inorganic mercury and organic mercury. Metallic Hg is the main form of mercury released into the air by natural processes. Many organic and inorganic compounds can be formed from the mercuric cation. Inorganic Hg compounds occur when Hg combines with elements such as chlorine, sulfur and oxygen. Chromium is one of the heavy metal pollutants produced from ferrochrome, tanning and pigment industries. Chromium in nature exists in toxic Cr^{+4} and Cr^{+3} forms. The Cr^{+6} ion is known to affect several plant processes (7). Cr^{+3} is readily oxidized to Cr^{+6} in soils because of the presence of oxidized Mn, which serves as the electron acceptor in the reaction. Heavy metals can be absorbed from the soil and atmosphere, accumulate in the organs of the plants and show their phytotoxic effects.

Cytoplasmic and wall bound peroxidase and IAA oxidase activities were studied in hypocotyls of *Phaseolus vulgaris* L. seedlings treated with Hg or Cr.

Materials and methods

Seeds of *Phaseolus vulgaris* L. were soaked in tap water for 3 h. They were then thoroughly washed with tap water, followed by distilled water. Then they were transferred to moistened filter paper for germination in darkness for 24 h. Uniformly germinated seeds were transferred to sieve culture dishes (190 x 50 cm, mesh size 3 mm x 3 mm) containing nutrient media (8) and distilled water or Hg or Cr. Seedlings treated with distilled water served as the control. Two concentrations of Hg (0.05 mM - T1 and 0.4 mM - T2) and 2 concentrations of Cr (0.5 mM - T1 and 1.0 mM - T2) were selected for experimental study. These concentrations were selected from a range of concentrations for Hg (0.01 mM to 0.4 mM) and for Cr (0.01 mM to 5.0 mM), tested in pilot/trial experiments. The seedlings were allowed to grow at 25 + 1 °C in a light room where continuous illumination was provided by 6 fluroscent tubes 0.5 m high (ca. 200 mM $m^{-2}s^{-1}$). The growth medium was renewed every 24 h. The time when the sieve culture dishes were transferred to the light room was taken as 0 h. After 12 h, the hypocotyls were demarcated into 2 segments with India ink. The segment near the root was designated as the lower segment and that near the cotyledon was designated as the upper.

Growth analysis

After an interval of 8 h (initially) or 24 h (later), 20 seedlings from each treatment were selected for growth analysis. The hypocotyls were cut into their respective segments and their lengths were measured to the nearest millimeter and their fresh weight recorded. In the present work, the lower segment did not show any growth in any treatment in any period in the presence or absence of Hg or Cr. Therefore, only the data for the upper segment are presented; the hypocotyl means the upper segment only. Similar results were also reported in our earlier studies (9,10).

Biochemical analysis

The required numbers of hypocotyls (300 mg) were separated, chilled and then homogenized in a pre-chilled mortar and pestel with a pinch of sand in K-phosphate buffer (0.02 M, pH 6.4). The mixture was centrifuged at 10,000 x g for 10 min. The supernatant was used for estimating cytoplasmic peroxidase and IAA oxidase activities. The residual wall material after the extraction of soluble cytoplasmic enzymes was thoroughly washed with distilled water and centrifuged until the washings were free of peroxidase reaction with guaiacol. The wall fraction was then kept with 10 ml of 1 M NaCl for 1 h at room temperature with regular shakings to release (ionically) bound enzymes. After centrifugation at 10,000 x g for 10 min, the supernatant served as a source of wall bound enzymes.

Peroxidase assay

Peroxidase activity was measured by recording changes in absorbance at 400 nm (ΔA400), following the method described by George (11) and Maehly (12). The various hydrogen donors used were ferulic acid, caffeic acid, pyrocatechol and pyrogallol. The brown color that developed due to the oxidation of different hydrogen donors in the presence of H_2O_2 was spectrophotometrically measured. The assay mixture consisted of 12 mM K-phosphate buffer (pH 6.4), 4 mM hydrogen donors, enzymes and 1 mM H_2O_2. The activity is expressed as ΔA400 g fresh $weight^{-1}$ min^{-1}.

IAA oxidase assay

IAA oxidase activity was determined by a modified version of the method described by Gordon and Weber (13). The assay mixture consisted of 0.16 mM 2-4 dichlorophenol, 0.2 mM $MnCl_2$, 8 mM K-phosphate buffer (pH 6.4), 0.2 mM IAA and 1 ml of enzyme extract. The activity was estimated as described earlier (14). Both assays were optimized for linearity with time and protein concentration. The complete biochemical analysis was performed in 3 independent preparations and the mean values are presented.

Results and Discussion

Initially, both Hg and Cr were used at 0.5 mM and 1.0 mM levels for the sake of uniformity, but even 0.5 mM Hg was highly toxic, making some of the observations difficult, and the Hg concentration was reduced to 0.4 mM. Hence the concentrations selected for Hg were 0.05 mM (T1) and 0.4 mM (T2) and for Cr 0.5 mM (T1) and 1.0 mM (T2).

Changes in the root length as affected by Hg or Cr are shown in Figure 1. Initially, the root length of both heavy

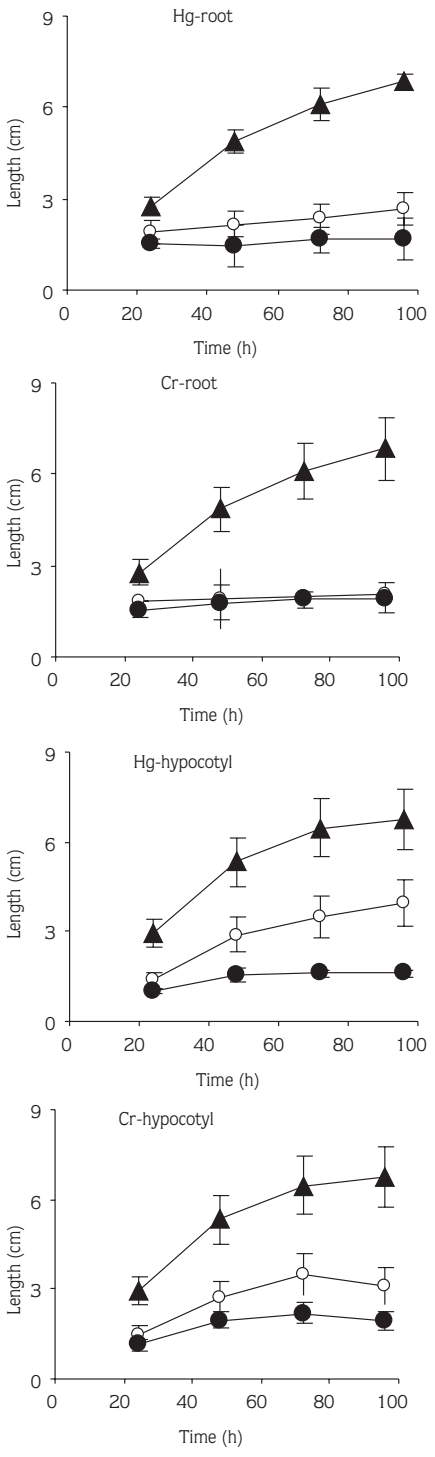

Figure 1. Effect of different concentrations of mercury and chromium on root and hypocotyl length in phaseolus seedlings. (Δ) DW control, (○) T1 and (●) T2. Vertical bars represent ± standard deviation. (a) Mercury root; (b) mercury–hypocotyl; (c) chromium–root; (d) chromium–hypocotyls.

metal treated seedlings was small. In the controls a steady increase in root length was observed, while in treated seedlings great inhibition was noted. The inhibition was greater in Cr treated seedlings. As seen in the Figure, both heavy metals affected root growth from the initial stage, i.e. at 24 h. There was reduction or inhibition of the growth of the main axis of the root with a consequent reduction in root length.

Changes in hypocotyl length in both Hg and Cr treated seedlings are shown in Figure 1. Both concentrations of Hg and Cr inhibited hypocotyl length. Initially, even at 24 h hypocotyl growth inhibition was evident. The inhibition was slightly higher in Cr treatment than in Hg treatment. Another distinguishing feature was that among the concentrations used, in Hg treatment the difference between T1 and T2 was more prominent when compared with that between T1 and T2 in Cr treated hypocotyls. This inhibition may be attributed to inhibition of cell division in apical meristems (root as well as hypocotyl), which disrupted the metabolic pathway responsible for elongation growth. Inhibitory effects of heavy metals on plant growth and physiological processes have been reported (15,16).

Peroxidase activity was higher in both Hg and Cr treated hypocotyls (Figures 2-5). A similar increase in peroxidase activity in Cd and Pb treated pine needles was reported by Durmus and Kadioglu (17). In both Hg and Cr treated seedlings, more cytoplasmic peroxidase activity was determined with caffeic acid and pyrogallol as hydrogen donors, while when ferulic acid and pyrocatechol were used less activity was determined (Figures 2, 3). A slightly different trend was observed with wall bound peroxidase activity. Wall bound peroxidase activity with all 4 hydrogen donors was higher in Cr - treated seedlings than in Hg treated seedlings (Figures 4, 5). However, maximum wall bound peroxidase activity in both Hg and Cr treated seedlings was with caffeic acid, and the minimum was with pyrocatechol. A clear inverse effect with hypocotyl elongation was seen.

Other stresses like uv radiation, pathogens, low temperature, drought and heavy metals, induce peroxidase activity to provide cells with resistance against the formation of H_2O_2 (18,19). In plants, peroxidases protect cells against harmful concentrations of hydroperoxides (20). Peroxidases are of importance in a

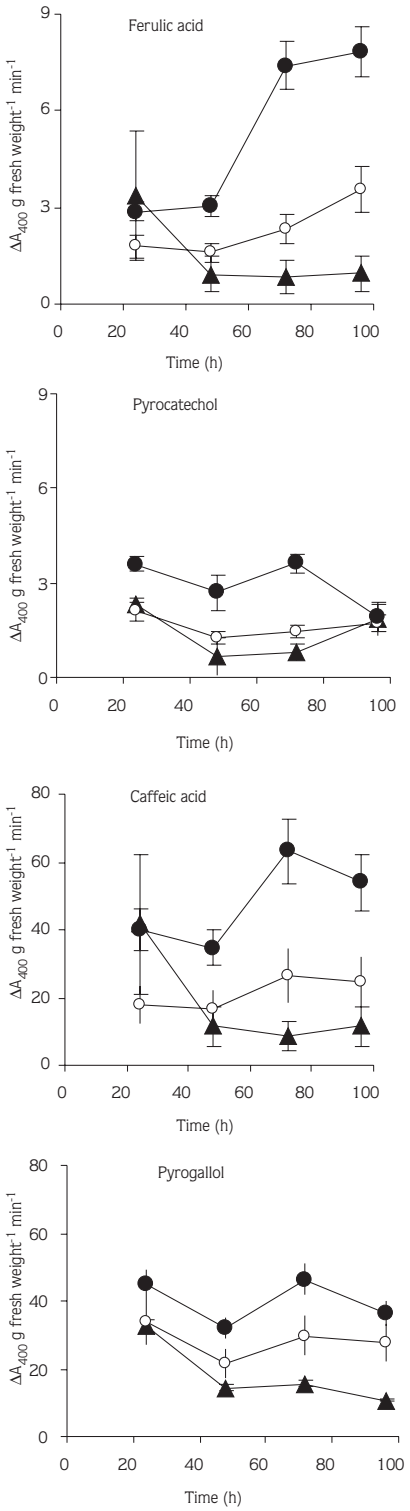

Figure 2. Changes in cytoplasmic peroxidase activity with 4 hydrogen donors in mercury treated phaseolus seedlings. (Δ) DW control, (ↄ) T1-0.05 mM and (●) T2-0.4 mM. Vertical bars represent ± standard deviation.

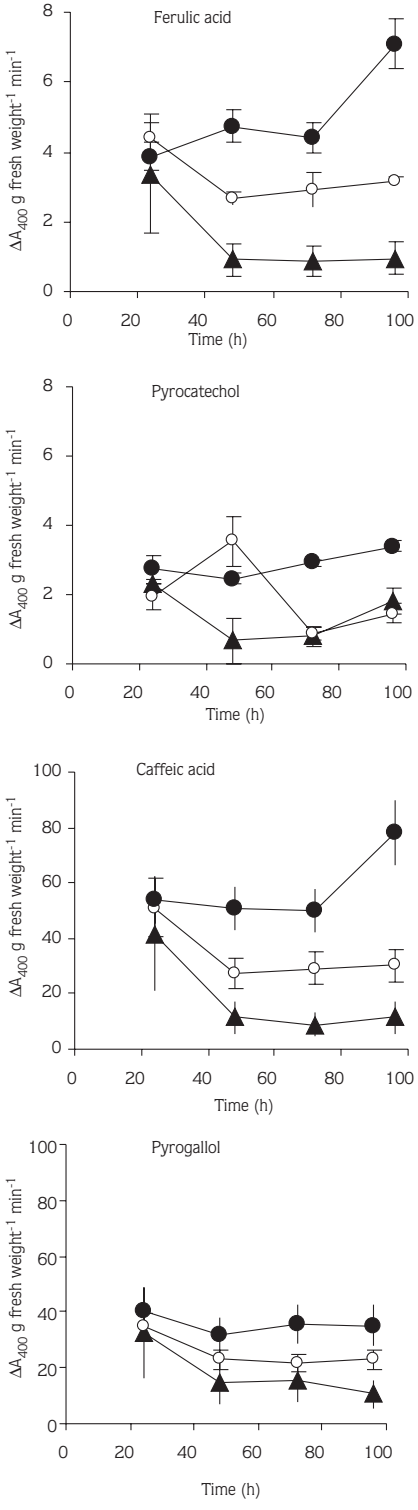

Figure 3. Changes in cytoplasmic peroxidase activity with 4 hydrogen donors in chromium treated phaseolus seedlings. (Δ) DW control, (ↄ) T1-0.5 mM and (●) T2-1.0 mM. Vertical bars represent ± standard deviation.

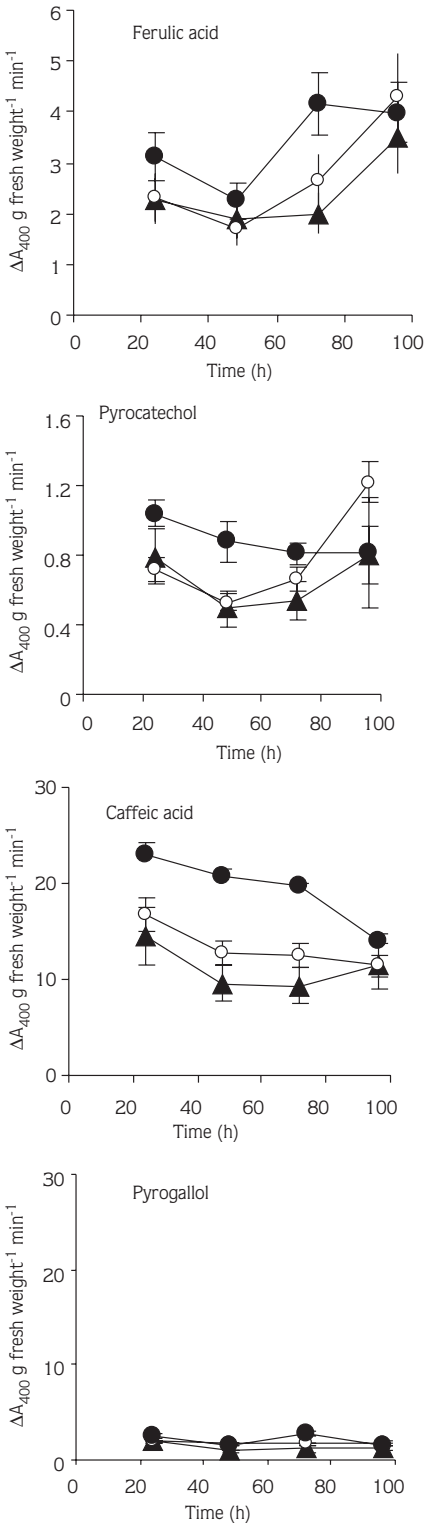

Figure 4. Changes in wall bound peroxidase activity with 4 hydrogen donors in mercury treated phaseolus seedlings. (Δ) DW control, (○) T1-0.05 mM and (●) T2-0.4 mM. Vertical bars represent ± standard deviation.

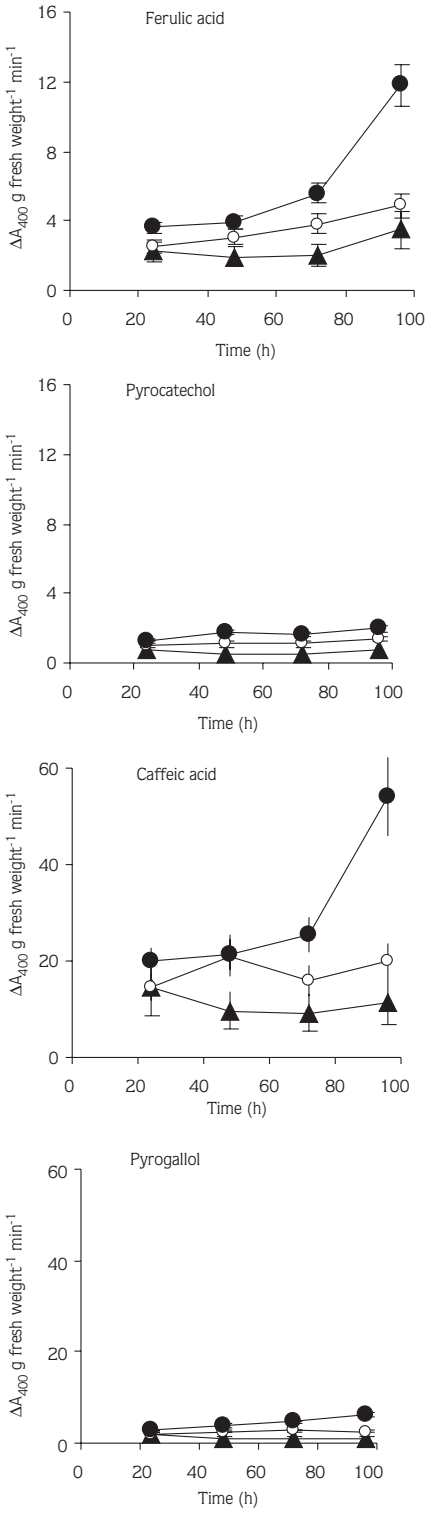

Figure 5. Changes in wall bound peroxidase activity with 4 hydrogen donors in chromium treated phaseolus seedlings. (Δ) DW control, (○) T1-0.5 mM and (●) T2-1.0 mM. Vertical bars represent ± standard deviation.

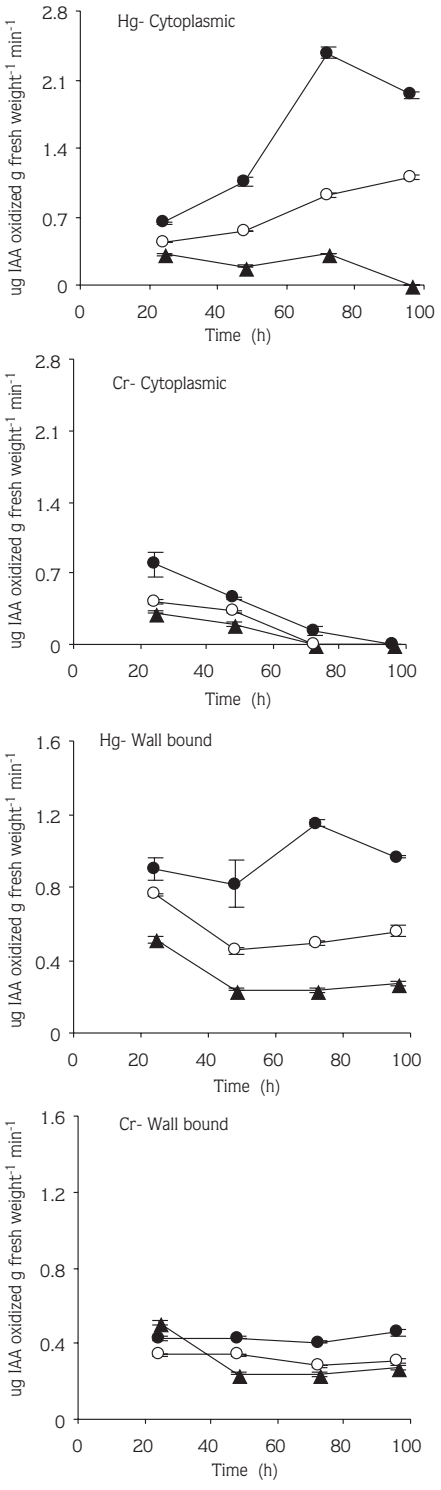

Figure 6. Effect of different concentrations of mercury and chromium on IAA oxidase activity in phaseolus seedlings. (Δ) DW control, (○) T1 and (●) T2. Vertical bars represent ± standard deviation. (a) Mercury–cytoplasmic; (b) mercury–wall bound; (c) chromium–cytoplasmic; (d) chromium–wall bound.

variety of cellular functions. The existence of an inverse correlation between cell wall peroxidase and growth is well documented (3,21,22). The high peroxidase activity observed in the present study in hypocotyls of seedlings treated with both heavy metals (Hg or Cr) might indicate the initiation of a disruption in the biochemical processes that precede the appearance of visible symptoms of toxicity. Increase in lipid peroxidation in Cr treated wheat seeds due to the production of free radicals by Cr mediated reactions has been reported (23).

Changes in IAA oxidase activity are shown in Figure 6. In the controls, the IAA oxidase activity was very much lower. In Hg treated seedlings a clear concentration effect was observed. The control seedlings had minimum IAA oxidase activity and maximum length while T2 had maximum activity and minimum length. In Cr treatment, both concentrations had slightly higher activity (cytoplasmic and wall bound) than that of the control seedlings. A close parallelism between peroxidase activity with different hydrogen donors and IAA oxidase activity supports the view that the ability to oxidize IAA is one of the many capabilities intrinsic to the peroxidase enzyme molecule. Thus it may be concluded that peroxidase restricts elongation growth by the formation of diphenyl cross-links while IAA oxidase may play a role in the fine regulation of the cellular level of IAA. It appears that Hg is more toxic than Cr because a lower concentration of Hg (0.4 mM) induced the same amount of growth inhibition as a higher concentration of Cr (1 mM). Seedlings did not survive when Hg concentration exceeded 0.4 mM while the same seedlings survived in a 1 mM Cr concentration.

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