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BRIEF COMMUNICATION

Mercuric chloride induced membrane damage in tomato cultured cells

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Abstract

Suspension cultures of tomato cells were used to study the membrane injury by the toxic concentration of mercuric chloride. Assessment of electrolyte leakage, UV-absorbance of the tissue leachates, relative leakage ratio, injury index, membrane lipid peroxidation, lipoxygenase activity, α-amino nitrogen and total soluble carbohydrate contents showed the extent of membrane damage as a function of the increasing concentration of mercuric chloride. It is suggested that the selected parameters can be used as qualitative tests for determination of stress-induced membrane damage.

Additional key words: lipoxygenase, *Lycopersicon esculentum,* malon dialdehyde, relative leakage ratio.

It has been found that toxicity imposed by heavy metals involves an overall disruption in the synchronization of different metabolic processes occurring in the cells, the degree and extent of injury being dependent on the concentration of the metal present. Of all the toxic heavy metals, mercury seems to be the most potent pollutant basically affecting photosynthesis (Nag *et al.* 1981). Stress-induced membrane damage is very much related to membrane lipid peroxidation, that is caused by free radicals (Dhindsa and Matwe 1981, Cakmak and Horst 1991). The free radicals and H_2O_2 are major sources of active oxygen species, such as superoxide ($O_2^{\cdot}$), hydroxyl radical (OH^-), and singlet oxygen ($^1O_2$). These active oxygen species, in turn, interact with protein and lipid components of membranes, causes damage (Greger and Lindsberg 1986). Lipoxygenase (LOX) mediates poly-unsaturated fatty acids (PUFA) oxidation in membranes and produces free radicals

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Abbreviations: LOX - lipoxygenase; PUFA - poly-unsaturated fatty acids; MDA - malon dialdehyde; EC - electric conductivity; 2,4-D - 2,4-dichlorophenoxy acetic acid; RLR - relative leakage ratio; α-NH_2 -α-amino nitrogen; TBA -thiobarbituric acid; d.m. - dry mass.

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which in turn cause membrane destruction. It has also been shown that malon dialdehyde (MDA), which is one of the decomposition products of PUFA of biomembranes showed greater accumulation under salinity treatment (Choudhury and Choudhuri 1993). Production of these highly active free radicals through LOX activity under different abiotic stresses are well documented in literature (Heath and Packer 1968, Kastori and Petrovic 1992). But reports regarding the membrane destruction by free radicals under $HgCl_2$, stress, especially in *in vitro* cultured cells, are surprisingly scanty; although the maintenance of integrity of cell membrane is an important factor for tolerance to heavy metals. The aim of the present study is to determine the effect of $HgCl_2$ on membrane damage of tomato cell cultured *in vitro*.

Surface sterilized seeds of *Lycopersicon esculentum* Mill. were throughly washed with sterile distilled water. Embryo derived callus cultures were the basic starting materials for cell suspension cultures and those were maintained in the standard MS medium (Murashige and Skoog 1962) with 15 % coconut milk, 2 % saccharose and 1 mg dm^{-3} 2,4-D. Cell suspension cultures were maintained on a rotary shaker in an environmental chamber at temperature 25 ± 2 °C, photoperiod 12 h, irradiance 270 μmol m^{-2} s^{-1}) and were routinely transfered each 7th day by adding 5 cm^3 of cultures to 30 cm^3 of fresh culture medium. The day of transfer was designated as zero day. Cultures in mid-log phase were treated with $HgCl_2$ (5, 25, 50, 250, 500, 750 μM) for 8 h. UV-absorbing substances were determined using methods of Redmann *et al.* (1986) with some modifications. Cultured cells (300 mg) were placed in 1.5 cm^3 of sterile distilled water and placed on a rotary shaker for 24 h in order to rinse exhaustively. At the end, 1.5 cm^3 of the bathing solution from each flask was taken out and absorbance at 280 nm (A_{280}) was recorded. Then the aliquot was again placed back to each of the mother flasks and the flasks with bathing solutions and cultured cells were autoclaved at 1 kg cm^{-2} pressure for 15 min to destroy the integrity of the membrane. Now the second aliquot of 1.5 cm^3 from each of the flasks was taken for A_{280} measurement. The relative leakage ratio (RLR) of the UV-absorbing substances was calculated as the ratio: A_{280} (before autoclaving)/A_{280} (after autoclaving). The absorbance spectra of electrolyte leakage were scanned using a scan speed of 750 cm s^{-1} in the wavelength range between 250 - 300 nm. Leakage of electrolyte (measure of membrane permeability), α-amino nitrogen (α-NH_2) and soluble carbohydrates in the leachate were estimated following the method of Bhattacharjee and Mukherjee (1995), respectively. For membrane injury index, 300 mg of cultured cells were placed in glass vial containing 15 cm^3 of deionized water and incubated at 25 ± 2 °C for 24 h. The electric conductivity (EC) of the bathing medium was measured at 25 °C by a conductivity meter. The cells with leachate were then autoclaved and EC measured again. The injury index was calculated using the formula (Sullivan 1972):

$$\text{injury [\%]} = [1-(1-T_1/T_2)/(1-C_1/C_2)] \times 100$$

where C_1 and C_2 are EC of control sample before and after autoclaving and T_1 and T_2 are EC of $HgCl_2$ treated samples before and after autoclaving. To estimate MDA content, thiobarbituric acid (TBA) test was performed (Heath and Packer 1968). For the extraction and estimation of LOX activity, the method of Peterman and Siedow (1985) was followed.

Maximum UV-absorbance of the leachate was recorded between 270 and 280 nm and the absorbance gradually increased with the increasing concentration of $HgCl_2$ (Fig. 1). Also, Rauser and Hansen (1965) have shown the leakage of UV-absorbing substances from soybean roots due to specific ion effects of the treatment solutions.

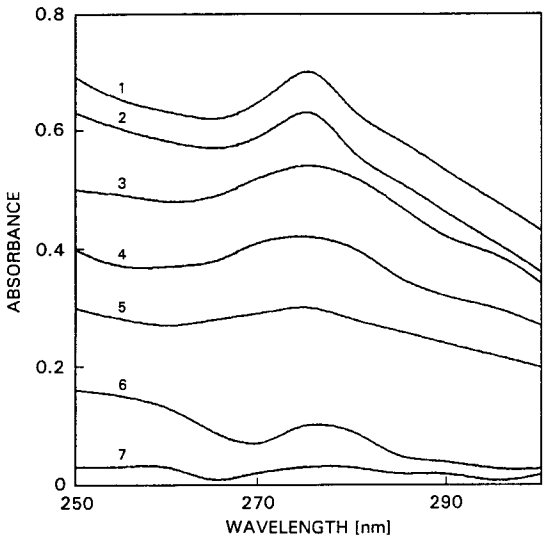

Fig. 1. Absorption spectra of the bathing medium of $HgCl_2$ treated and untreated tomato cells cultured *in vitro* (*1* - untreated cells, *2, 3, 4, 5, 6* and *7* - cells treated with 5, 25, 50, 250, 500 and 750 μM $HgCl_2$, respectively).

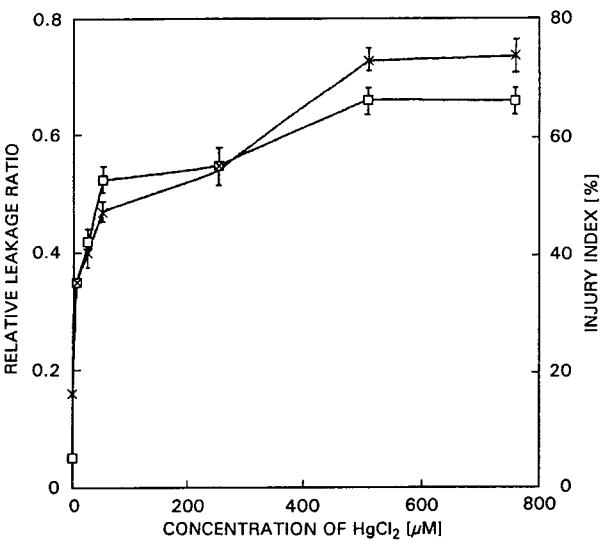

Fig. 2. Effect of different concentrations of $HgCl_2$ on relative leakage ratio (RLR - *crosses*) and injury index (*squares*) of tomato cells cultured *in vitro*. *Bars* represent ± SE.

The data of injury index (Fig. 2) showed the extent of membrane damage as a function of the increasing concentration of $HgCl_2$. Another criterion of membrane injury, RLR, showed the same type of increase as found in case of injury index. The membrane damage correlated with $HgCl_2$ concentration. MDA level also increased proportionally with the rising concentration of $HgCl_2$ (Fig. 3). Proportional to the increasing $HgCl_2$ concentration, the activity of LOX increased quite significantly, being highest at 500 μM $HgCl_2$ treatment. Richard *et al.* (1991) and Dhindsa and Matwe (1981) showed similar increase of LOX activity due to salinity stress. Thus, the enhanced activity of LOX along with the increased accumulation of MDA with the increasing salinity might provide a plausible clue to increasing membrane injury of our experimental material. Tissue permeability, as envisaged by leakage of cellular substances in the bathing medium, also significantly increased with increasing $HgCl_2$ concentration (Table 1). The assessment of EC, A_{280} value, α-NH_2 and soluble carbohydrates shows that in all the cases rising concentration of $HgCl_2$ was

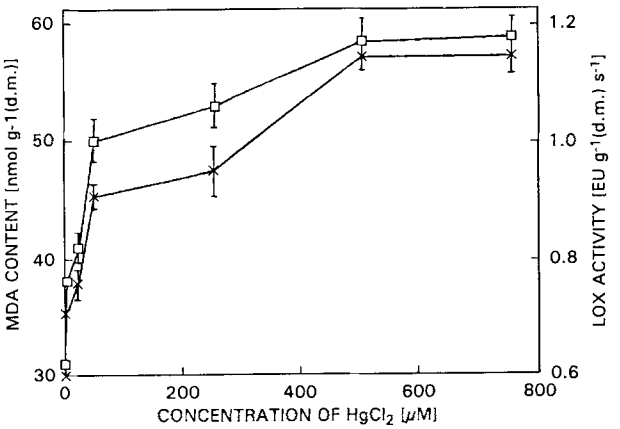

Fig 3. Effect of different concentrations of $HgCl_2$ on membrane lipid peroxidation (MDA content - *crosses*) and lipoxygenase activity (LOX - *squares*) in tomato *in vitro* cultured cells. *Bars* represent ± SE.

Table 1. Influence of different concentrations of $HgCl_2$ on electric conductivity (EC) of the bathing medium, A_{280} value, α-NH_2 and soluble carbohydrate content in leachate of tomato cultured cells. Standard error is given as ± mean value.

$HgCl_2$ [μM]	EC [mS cm^{-1} g^{-1} (d.m.)]	A_{280}	α-NH_2 [mg g^{-1} (d.m.)]	Sol. carbohydrates [mg g^{-1} (d.m.)]
0	1.00 ± 0.02	0.16 ± 0.001	0.06 ± 0.01	15.28 ± 0.12
5	8.60 ± 0.02	0.31 ± 0.001	0.45 ± 0.09	29.91 ± 0.21
25	9.60 ± 0.02	0.38 ± 0.002	0.58 ± 0.04	27.83 ± 0.65
50	11.30 ± 0.01	0.45 ± 0.003	0.74 ± 0.03	30.39 ± 0.09
250	11.90 ± 0.02	0.48 ± 0.002	1.27 ± 0.02	33.29 ± 1.15
500	14.00 ± 0.01	0.57 ± 0.002	1.47 ± 0.06	34.66 ± 1.11
750	14.30 ± 0.02	0.58 ± 0.001	1.45 ± 0.05	34.01 ± 1.18

responsible for the maximum efflux of the membranes out of the tissue confirming unequivocally the membrane injury and leakage as a function of the increasing $HgCl_2$ concentration.

Data presented here clearly revealed that $HgCl_2$ caused significant membrane damage in tomato cultured cells as manifested by electrolyte leakage, increased amount of α-NH_2, soluble carbohydrates and A_{280} value, increasing injury index and LOX activity in case of almost all the concentrations of $HgCl_2$ used. This leakage is proportional to the extent of $HgCl_2$ stress *i.e.* increasing concentration.

Thus, the extent of stress-influenced membrane injury can be assessed easily by the above-mentioned biochemical estimations.

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