EFFECT OF MERCURIC CHLORIDE ON GROWTH AND CYTOTOXICITY OF SOYBEAN Glycine max (L.) Hepper

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Abstract
The present experiment was undertaken to determine the growth and cytomorphology of soybean under Mercuric Chloride (HgCl$_2$) to determine the cytotoxicity effects of different concentrations (Control, 1.0, 2.0, 3.0, 4.0 and 5.0 ppm) of HgCl$_2$. In germination studies, the morphological parameters such as germination percentage, root length, shoot length; fresh weight and dry weight of seedlings were decreased with increasing dose of HgCl$_2$ concentrations. There is no germination of Glycine max L. seeds was recorded at 5.0 ppm HgCl$_2$ concentration. Chromosome aberration assay was used to determine the mitotic indices and rate of chromosome aberration in Glycine max L. root tip cells due to HgCl$_2$ treatment. The results showed that the mitotic indices were complicated due to different concentrations of HgCl$_2$. However, the increase in HgCl$_2$ concentration has led to increase in the percentage of chromosomal aberration and mitotic index. The chromosome length, absolute chromosome length and average chromosome lengths were gradually found to decrease and considerable change in 2n number of chromosome with the increase in HgCl$_2$ concentrations. It is concluded that the HgCl$_2$ has significant cytological effect on the root tip cells. While higher concentrations of HgCl$_2$ were found to be more mutagenic and cytotoxic effect of soybean plants.

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Keywords: Mercuric Chloride, Glycine max (L.) Hepper, Germination percentage, Cytomorphology.

INTRODUCTION
Foremost environmental problems caused by industrialization are the increment in the concentration of heavy metals in the air, land and water. Mercury chloride HgCl$_2$ is important non-degradable toxic heavy metal pollution and increasing rapidly in our biosphere and is accumulated by plants in polluted areas. Human activities are estimated to account of 2 ×10$^4$ to 7×10$^4$ tones of mercury per year being released into the atmosphere and water supply [1]. It is a potential inhibitor of the algal growth [2]. HgCl$_2$ is not essential nutrients in higher plants, and the exposure to relative low concentrations results in serious toxicity [3]. One of the clearest phytotoxic symptoms induced by HgCl$_2$ is a diminution in plant growth, which is associated with disturbance of several metabolic processes, alteration of nutrient uptake and degeneration of cell ultrastructure [4]. Besides, the appearance of oxidative stress has well established [5]. The characteristics feature of heavy metal is poisoning and resulting in the inactivation of enzyme systems. All heavy metals are potentially toxic at elevated concentrations [6]. Although heavy metals form an essential part of human and plant nutrition, but their higher levels of plants uptake cause carcinogenic and mutagenic effects [7]. Early studies on organic mercury revealed the chromosome fragmentation, somatic mutations and pollen sterility [8]. Some workers observed toxic effects of mercury on germinating rich seeds [9] and on physiological aspects of the several legumes. The uptake of overload concentrations of heavy metals reduced the plants growth [10, 11]. This alternation in plant growth is correlated with the disruption of the physiological and cytological processes in plant cells. By this way the processes of respiration, photosynthesis and mitotic activity are greatly affected by the toxic effect of heavy metals [12]. There are a number of studies concerning the problem of the relationship between the increased heavy metal amount in nature and industrial environment, their mutagenic and carcinogenic effects and the increased cases of malignant tumour formations in man [13, 14, 15, 16, 17] and others discussed the problem of the relationship between the heavy metal amounts in natural and industrial environment, the increased frequency of chromosome mutations and the
Fig.1 Effect of Mercuric Chloride on Cytotoxicity of soybean Glycine Max (L.) Hepper
carcinogenic processes in the organism. Chromosomal rearrangements are one of the most frequently produced classes of mutation that result from the action of both physical and chemical mutagenic agents [18]. Our aim was to determine the effects of mercuric chloride on plant growth and mitotic index and the aberration rate of in chromosome soybean root tip cells.

MATERIALS AND METHODS
The soybean (Glycine max (L.) Hepper) seeds were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore. The seeds are uniform size; color and weight were selected for the experimental purpose. Mercuric chloride salt (Molecular weight 271.50), the different concentrations viz., Control, 1.0, 2.0, 3.0, 4.0 and 5.0 ppm of HgCl$_2$ solution were prepared freshly at the time of experiments. Uniform size of healthy soybean seeds were equispacially arranged in sterilized petriplates lined with filter paper (Whatman No. 1) in 12 cm diameter Petri plates. The seeds were treated with equal volumes (25 mL) of different concentrations (1.0, 2.0, 3.0, 4.0 and 5.0 ppm) of HgCl$_2$ solution. One set of seeds irrigated with distilled water was maintained as the control.

Root tip meristems were obtained from germinating seeds in petridishes. Both the experimental and control root were excised from the seeds, and fixed in ethanol-acetic acid (3:1) for cytological studies. The root tip squashes were made, haematoxylin squash technique of [19]. The root tips were hydrolysed in 0.1 N HCl for 15 to 20 min at 60°C and then they were thoroughly washed in distilled water and treated with 4% iron alum (Ferric ammonium sulphate) for 5 min. The root tips were then washed thoroughly in distilled water and transferred in dilute haematoxylin stain and kept for 3 hours. The root tips were thoroughly washed in distilled water and then they were treated in 45% acetic acid for a few seconds to soften the tissues. One or two root tips were placed on the tissues clean slide and squashed by using a cover slip and the slide was sealed and mounted. For each variable 5 root tip squashes were prepared and a minimum of 250-500 mitotic cells were counted from each slide.

Table 1: Effect of different concentrations of HgCl$_2$ (ppm) on growth parameters of Glycine max (L.) Hepper

<table>
<thead>
<tr>
<th>HgCl$_2$ Concentration (ppm)</th>
<th>Germination percentage</th>
<th>Shoot length</th>
<th>Root length</th>
<th>Fresh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>93.00 ± 3.72</td>
<td>11.30 ± 1.452</td>
<td>7.23 ± 0.289</td>
<td>0.912 ± 0.036</td>
</tr>
<tr>
<td>1.0</td>
<td>73.00 ± 2.93</td>
<td>9.21 ± 0.368</td>
<td>6.26 ± 0.250</td>
<td>0.784 ± 0.031</td>
</tr>
<tr>
<td>2.0</td>
<td>70.00 ± 2.80</td>
<td>7.73 ± 0.309</td>
<td>5.12 ± 0.204</td>
<td>0.634 ± 0.025</td>
</tr>
<tr>
<td>3.0</td>
<td>54.00 ± 2.16</td>
<td>6.36 ± 0.254</td>
<td>4.16 ± 0.174</td>
<td>0.434 ± 0.017</td>
</tr>
<tr>
<td>4.0</td>
<td>46.00 ± 1.84</td>
<td>3.38 ± 0.135</td>
<td>2.06 ± 0.094</td>
<td>0.348 ± 0.013</td>
</tr>
<tr>
<td>5.0</td>
<td>31.00 ± 1.24</td>
<td>2.48 ± 0.099</td>
<td>1.04 ± 0.041</td>
<td>0.200 ± 0.008</td>
</tr>
</tbody>
</table>

± Standard deviation

Table 2: Effect of different concentrations of HgCl$_2$ (ppm) on chromosomal changes in the root tips of Glycine max (L.) Hepper

<table>
<thead>
<tr>
<th>HgCl$_2$ Concentration (ppm)</th>
<th>Dry weight</th>
<th>Total chromosome length (µm)</th>
<th>Absolute chromosome length (µm)</th>
<th>Average chromosome length (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.368 ± 0.014</td>
<td>23.2 ± 0.963</td>
<td>11.2 ± 0.449</td>
<td>1.89 ± 0.075</td>
</tr>
<tr>
<td>1.0</td>
<td>0.298 ± 0.011</td>
<td>19.1 ± 0.764</td>
<td>10.0 ± 0.410</td>
<td>1.71 ± 0.068</td>
</tr>
<tr>
<td>2.0</td>
<td>0.204 ± 0.008</td>
<td>17.4 ± 0.696</td>
<td>8.1 ± 0.327</td>
<td>1.39 ± 0.055</td>
</tr>
<tr>
<td>3.0</td>
<td>0.163 ± 0.007</td>
<td>14.5 ± 0.580</td>
<td>7.0 ± 0.284</td>
<td>1.12 ± 0.048</td>
</tr>
<tr>
<td>4.0</td>
<td>0.108 ± 0.004</td>
<td>13.1 ± 0.451</td>
<td>6.6 ± 0.265</td>
<td>1.01 ± 0.045</td>
</tr>
<tr>
<td>5.0</td>
<td>0.074 ± 0.002</td>
<td>09.1 ± 0.364</td>
<td>6.1 ± 0.250</td>
<td>0.85 ± 0.034</td>
</tr>
</tbody>
</table>

± Standard deviation
Table 3. The effect of different concentrations of HgCl$_2$ (ppm) on total number of abnormal cells in *Glycine max* (L.) Hepper

<table>
<thead>
<tr>
<th>HgCl$_2$ Concentration (ppm)</th>
<th>Total number of cells analyzed</th>
<th>Total number of abnormal cells</th>
<th>Number of abnormal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>356</td>
<td>5</td>
<td>3 2 4</td>
</tr>
<tr>
<td>1.0</td>
<td>320</td>
<td>11</td>
<td>4 3 5</td>
</tr>
<tr>
<td>2.0</td>
<td>308</td>
<td>17</td>
<td>6 5 6</td>
</tr>
<tr>
<td>3.0</td>
<td>287</td>
<td>26</td>
<td>6 7 6</td>
</tr>
<tr>
<td>4.0</td>
<td>255</td>
<td>29</td>
<td>8 10 7</td>
</tr>
<tr>
<td>5.0</td>
<td>222</td>
<td>37</td>
<td>11 12 9</td>
</tr>
</tbody>
</table>

Table 4. The effect of different concentrations of HgCl$_2$ (ppm) Frequency of total abnormalities and mitotic abnormalities of *Glycine max* (L.) Hepper

<table>
<thead>
<tr>
<th>HgCl$_2$ Concentration (ppm)</th>
<th>Frequency of total abnormalities</th>
<th>% of Mitotic abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.65</td>
<td>0.98 0.68 0.86 0.72</td>
</tr>
<tr>
<td>1.0</td>
<td>5.72</td>
<td>1.92 1.24 1.54 1.02</td>
</tr>
<tr>
<td>2.0</td>
<td>8.52</td>
<td>2.86 2.00 2.12 1.88</td>
</tr>
<tr>
<td>3.0</td>
<td>10.88</td>
<td>4.98 4.56 3.65 2.55</td>
</tr>
<tr>
<td>4.0</td>
<td>13.62</td>
<td>6.74 5.80 4.86 2.96</td>
</tr>
<tr>
<td>5.0</td>
<td>16.84</td>
<td>7.80 6.92 5.60 3.12</td>
</tr>
</tbody>
</table>

RESULTS

The decline of germination percentage such as root length, shoot length, fresh weight, dry weight, total chromosome length, absolute chromosome length and average chromosome length of soybean (*Glycine max* (L.) Hepper) seedlings with increase of HgCl$_2$ concentrations are given in (Table 1 and 2). The higher seed germination (93.0 %), root length (7.23 cm/seedling), shoot length (11.30 cm/seedling), fresh weight (0.912 g/seedling), dry weight (0.368 g/seedling), total chromosome length (23.2 μm), absolute chromosome length (11.20 μm) and average chromosome length (1.89 μm) were observed in the root tips of control seedlings (Table 3 and 4). Similarly, the lower germination percentage (31.0 %), root length, (1.04 cm/seedling) shoot length (2.48 cm/seedling), fresh weight 0.200 g/seedlings), dry weight (0.074 g/seedlings) total chromosome length (9.1 μm), absolute chromosome length (6.1 μm) and average of chromosome length (0.85 μm) were observed at 5.0 ppm HgCl$_2$ concentrations. The frequency of total abnormalities (3.65, 5.72, 8.52, 10.88, 13.62 and 16.84) Mitotic abnormalities like viz., Bridge (7.80), laggards (6.92), Stickiness (5.60) and Binucleate cells (3.12) was observed in various concentrations (control, 1.0, 2.0, 3.0, 4.0 and 5.0 ppm) of HgCl$_2$ respectively (Fig.1). Among the cells are gradually increased from control to 5.0 ppm onwards. Heavy metals are of great interest for research purpose with respect to toxicological importance to human health, plants and animals [20]. Due to rapid industrialization, urbanization and intensive agriculture increasing contamination of heavy metals in soil has become a major concern. Excessive level of heavy metals in the soil environment adversely affects the germination of seeds, plant growth, alter the level of biomolecules in the cells and interfere with the activities of many key enzymes related to normal metabolic and developmental processes [21, 22, 23]. One of the clearest phytotoxic symptoms induced by heavy metals is a diminution in plant growth, which is associated with disturbance of several metabolic processes, alteration of nutrient uptake and degeneration of cell ultra structure [4]. Ionic toxicity may be the cause of drastic effects of heavy metal salts on seed germination or it could be due to osmotic effect [24]. Reduction of seed germination can also be attributed to the alterations of selection permeability properties of cell membrane [25]. Reduced root and shoot length in response to heavy metal has been reported by a number of investigators [26, 27, 28, 29, 30]. The reduction in seedling growth during stress may be due to low water potential, hampered nutrient uptake and secondary stress such as oxidative stress [30]. The reason for reduced seedling growth under metal treatment could be the reduction in meristematic cells present in this region and some enzymes present in cotyledons and endosperm. During seedling growth hydrolysis of food reserves takes place which is carried out by hydrolytic enzymes. Activities of hydrolytic enzymes might be affected and the food did not reach to the radicle and plumule leading to the reduction in seedling growth. Similar observations have been made by several authors under various stressful conditions including metal toxicity [31, 32, 33]. The reduction of chromosome length was observed at 1.0 to 5.0 ppm HgCl$_2$ concentrations onwards. Similar findings were observed in earlier reports in different heavy metals [34] the number of abnormal cells like viz., bridge, laggard, stickiness and Binucleate cells is increased from control plants and decreased at 5.0 ppm HgCl$_2$ concentrations. Several agents have been reported to cause chromosomal stickiness, including X-rays [35], gamma rays [36], temperature [37], herbicides [38] and some chemicals present in the soil [39, 40, 41]. [42, 43] reported that high lead, zinc, cadmium and copper concentrations inhibited the growth of vegetative organs in some plant species. [44] Reported that pollution with heavy
metal salts induced an increase in the frequency of intrachromosomal mutations. The results from the present study are in good agreement with them. [45] Observed a major change in the nucleus of the root tip in response to zinc. Fragments, laggards, bridges recorded during our study are in accordance with the results of [46] after using heavy metals and also it can be attributed to chromosomal stickiness. The higher concentration of potassium dichromate the higher frequency of aberrations in Vicia faba root tips [47]. [48, 49, 50] suggested that stickiness might be due to disturbances in the cytochemically balanced reaction. The high doses of heavy metal supply has a toxic effect on cell division attributes. Similar type of abnormalities is due to loss of microtubule of spindle fibres [51, 52]. The macronuclei observed at higher doses may originate from a lagging chromosome or from a chromosome fragment [38].

References
28. Tomulescu, I.M., E.M. Radoviciu, V.V. Merca and A.D. Tuduce: Effect of copper, zinc and lead and the


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