Effect of chemical mutagens on chromosomal behavior of Allium cepa L.

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Abstract: Effect of chemical mutagens was studied in mitotic cells of Allium cepa L. For this purpose, root tips were treated with saturated aqueous solution of parachlorobenzene, 0.2% EMS and 0.2 % freshly prepared aqueous colchicines solution for 4 hours to 9 hours. The study revealed a wide range of chromosomal abnormalities such as stickiness, laggards, chromosomal bridge, unequal separation, nuclear budding, multinuclear cells etc. The chromosomal abnormalities in different mitotic stages were calculated on mitotic index, frequency of phases and percentage of abnormalities in mitosis. The chromosomal abnormalities increase with increasing duration of treatment. The results showed that in 0.2% colchicine and 0.2% EMS treated cells more than 50% abnormalities were observed after 9 hrs of treatment. While in PDB maximum chromosomal abnormalities observed after 6 hrs of treatment. Maximum MI was recorded as 68% in controlled cells. While in PDB, colchicines and EMS treated cells minimum 9.5%, 20% and 23% MI were observed respectively after 9 hrs of treatment.

Keywords: Chromosome, Chromosome aberration, Mitotic index, Mutagens

Introduction
Different types of mutagens that may be physical or chemical are useful for creating variability which is important in plant improvement programme. The chemical mutagens are most effective and efficient on morphological, biochemical and cytological changes and that ultimately affect on yield and phenotype of the plants. Apart from the useful effect these mutagens have also their cytotoxic and genotoxic effect that can be estimated by the chromosomal abnormalities observed in mutagen treated plants. The degree of cytological abnormalities either in mitosis or meiosis is an useful criteria to estimate the effect of mutagen. Variety of nuclear and nucleolar abnormalities have been reported by many workers in different plant species by using mutagens. (Vadluri et al., 2016; Dorofeei & Bercu 2013; Ramesh 1983; Reddi and Reddi 1984). Joshi et.al. (2011) reported mutagen sensitivity in Onion (Allium cepa L.) and stated that low dose of mutagens could be suitable for creating variability. EMS is highly potential to cause point mutations in plants (Hajra, 1979) and leads to produce plants with different and new characteristics (Thurling and Depittayanan 1992). Cytological studies provide information about the response of various genotypes to mutagens and provide better chance of selection. Root tips of various plant species has been utilized to study the effect of mutagens. Mitotic index (MI) can be considered as a reliable method to determine the presence of cytotoxic effects of mutagens. Allium cepa L. is one of the most suitable plants for detecting different types of xenobiotics. The present investigation was carried out to ascertain the cytotoxic effect of mutagens and also to find out the potential of chemical mutagens on root meristem cells of Allium cepa L.

Materials and Methods
Healthy root tips of about 5 mm of Allium cepa L. are treated with saturated aqueous solution of PDB; 0.2% EMS and 0.2 % aqueous solution of colchicines for 4 to 9 hours. Then the root tips were washed thoroughly with distilled water to avoid residual effect and then fixed in Carnoy’s I fixative containing 3 parts of absolute ethanol and 1 part of glacial acetic acid for 24 hours. Acetorcein smear technique has been used by following the method of Sharma & Sharma, (1980). Two hundred (200) cells were scored to analyse mitotic index (MI) and expressed as a percentage. The Mitotic Index (MI) represents the total number of dividing cells in relation to the number of analysed cells. The frequency of chromosomal aberration was expressed as the number of aberrant cells per 100 dividing cells examined and microphotograph was taken at 1000x magnification.

Results and Discussion
Allium cepa L. is a diploid plant with eight pairs of relatively large chromosomes that allows for the easy detection of chromosomal aberrations. Mitosis of control plant showed 2n = 16 normal chromosomes during metaphase. The results showed treatment duration dependent Mitotic Index (MI). In controlled cells it was 68% and it
decreases with increase duration of mutagens treatments (Table-1). Minimum MI was recorded as 9.5%, 20% & 23% in 9 hrs treatment of PDB, colchicines and EMS respectively. Hilada et al. (2017) reported the mutagenic effect of Alprazolam which causes decrease in MI in onion cells. Decrease in the mitotic index (MI) can be considered as a reliable method to determine cytotoxic effect of mutagen. The changes in MI of *Allium cepa* L. cells are indicators of cytotoxic and genotoxic potential and mitodepressive activity of mutagens. All the mutagens were capable of inducing various types of chromosomal abnormalities in the stages of mitosis and showed highly cytotoxic effect. Chromosomal aberrations (CAs) include either in the changes of total number or changes in orientation of chromosomes. In PDB treated cells, high percentage of chromosomal abnormalities observed after 6 hours of treatment which was recorded as 53%. While in colchicines and EMS treated cells 6 hours treatment was not that much effective and nine (9) hrs treatments showed highest percentage of abnormalities and it was recorded as 55% and 58% respectively (Table 1). The commonly observed chromosomal abnormalities were chromosomal stickiness, chromosome loss, chromosome breakage, anaphase bridge, laggard, multinuclear condition, fragmented nucleus disturbed polarity, delayed nuclear membrane formation etc. Natarajan (1958) and Siddiq (1964) reported the presence of multinuclear condition and nuclear budding in mutagen treated maize and sorghum. Multinuclear condition may be due to disruption and dispersion of RNA and indicating the formation of adventitious nucleus. Occurrence of lagging chromosome may be due to abnormal spindle formation and chromosomal breakage. Precocious separation of chromosome in the treated cells may be probably due to spindle disfunction. Roy et al., (1971) observed precocious separation of chromosome in gamma irradiated *Curcumas sativus*. Stickiness of chromosome was probably due to the interference in the formation of telomeres and may fuse with another broken chromosome ends. The spindle disturbance and polar deviation can lead to abnormal kinetics of chromosome and prolonged prophase. Chromosome bridge was common occurrence in all the treatments and is useful for obtaining information on clastogenic activity. The presence of dicentric chromosomes and unequally exchanged chromatids undergoing translocation may be the probable cause of Chromosome Bridge at anaphase. The presence of fragmented nuclei and polynuclear cells may lead to aneuploidy and then to cell death (Hilada et al., 2017).

![Image of chromosomal abnormalities](image_url)

**Figure.** A- Late Prophase; B – Normal Metaphase; C- Normal Anaphase; D - Stickiness; E- mitotic pairing; F -H- Unequal separation; I-K –Laggard; L- delayed nuclear membrane formation; M – polyploid cell; N- Disturbed Polarity; O&P –Chromosome bridge; Q- Delayed Prophase; R-T Multipolarity.

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Table 1. Chromosomal behavior of Mutagen treated *Allium cepa* L.

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Duration of treatment (hrs.)</th>
<th>Mitotic Index (%)</th>
<th>Chromosomal Abnormalities (%)</th>
<th>Stickiness (%)</th>
<th>Laggard (%)</th>
<th>Bridge (%)</th>
<th>Unequal separation (%)</th>
<th>Movement of Chromosomes Toward one pole (%)</th>
<th>Polyploidy cell (%)</th>
<th>Multinuclear condition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDB</td>
<td>4</td>
<td>62</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Saturated solution)</td>
<td>6</td>
<td>44</td>
<td>53</td>
<td>9.6</td>
<td>-</td>
<td>8.2</td>
<td>9.4</td>
<td>10.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Colchicine</td>
<td>6</td>
<td>34</td>
<td>28</td>
<td>5.1</td>
<td>6.3</td>
<td>2.7</td>
<td>6.1</td>
<td>-</td>
<td>-</td>
<td>7.8</td>
</tr>
<tr>
<td>(0.2%)</td>
<td>9</td>
<td>20</td>
<td>55</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11.7</td>
<td>12.2</td>
<td>-</td>
</tr>
<tr>
<td>EMS</td>
<td>4</td>
<td>53</td>
<td>13</td>
<td>7.3</td>
<td>5.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(0.2%)</td>
<td>6</td>
<td>38</td>
<td>32</td>
<td>-</td>
<td>9.0</td>
<td>-</td>
<td>14.6</td>
<td>6.4</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>23</td>
<td>58</td>
<td>17.7</td>
<td>9.9</td>
<td>8.2</td>
<td>11.6</td>
<td>8.7</td>
<td>-</td>
<td>1.9</td>
</tr>
</tbody>
</table>

**Conclusion**

The result of the present investigation indicated that prolong treatment of PDB, colchicines and EMS have cytotoxic and genotoxic effect. Prolong treatment with these mutagens cause chromosomal aberrations nuclear alterations and spindle disturbance which may affect on the cell cycle. The significant inhibition of mitotic index can affect on cell proliferation and ultimately on the growth of an organism.

**References**

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