Studies on the Effect of Maleic Hydrazide on Root Tip Cells and Pollen Fertility in Trigonella foenum-graecum L.

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Abstract: In the present investigation, the effect of maleic hydrazide (MH) has been studied on seed germination, mitosis, and pollen fertility of Trigonella foenum-graecum L. to test its cytotoxicity. Six different concentrations (0.01%, 0.02%, 0.03%, 0.04%, 0.05%, and 0.06%) of maleic hydrazide (MH) were given at seed level. The abnormalities noticed were both physiological and clastogenic. Fragments, stickiness of chromosomes, univalents, multivalents, and anaphase bridges were the most frequent abnormalities. The decrease in seed germination and pollen fertility was found significant at 0.05 level. Mitotic index reduced gradually as the concentrations of Maleic Hydrazide increased and it was found significant at 0.01 and 0.05 levels.

Key Words: Cytotoxicity, Fenugreek, Maleic Hydrazide (MH), Mitotic Index (MI)

Introduction

Fenugreek (Trigonella foenum-graecum L.), an annual legume cash crop of India (Fazli & Hardman, 1978), commonly known as “methi”, is cosmopolitan in distribution, believed to be native to the Mediterranean region, and now widely cultivated in most parts of the world (Acharya et al., 2006). It can also be efficiently incorporated into short-term crop rotations in western Canada (Moyer et al., 2003). Fenugreek has been reported to generate a high yield of good quality forage and hay or silage (Mir et al., 1998). In addition, the small inconspicuous flowers from fenugreek had a 4% success rate when crossed (Choudhury & Singh, 2001), making hybridization a less attractive route for crop improvement. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement (Acharya et al., 2007). Some legume crops that have been improved through mutation breeding are soybeans, string beans and French beans (Sigurbjornsson & Micke, 1974); navy pea beans and haricot beans (Sigurbjornsson, 1983); peas and fenugreek (Petropoulos, 1973, 2002); and lupines (Gaul, 1961). A majority of induced mutations in these plants are recessive, and can be observed to segregate in a 3:1 ratio in diploid crops like fenugreek (Gaul, 1961; Petropoulos, 1973; Singh & Singh, 1974).

Maleic hydrazide (1, 2-dihydro-3, 6-pyridazinedione) has been introduced into agriculture as a major commercial herbicide and a depressant of plant growth. The extensive use of herbicides in agriculture and their potential carcinogenicity strongly suggest the need to extend the cytotoxic evaluation of these compounds by using different methods. Here we report a cytotoxic evaluation of the herbicide maleic hydrazide in root tip cells and on pollen fertility.

Mode of action of maleic hydrazide presumably involved interference with sulphhydryl groups which reduced the synthesis of those enzymes in which SH-groups were required. Therefore, this chemical affects SH-bond in some way as a consequence of which the proteins necessary for the formation of spindle apparatus are also affected (Khan, 1997). Chromosome breakage effects of MH in plants were first described by Darlington and McLeish in 1951. MH also resulted in the inhibition of spindle formation and chromosome breakage during...
mitosis in root tips of onion and barley (Kaul & Choudhary, 1975).

Maleic hydrazide is a pyridazine that inhibits the synthesis of nucleic acids and proteins (De Marco et al., 1992). It is a well-known clastogenic agent in plants (Swietlinska & Zuk, 1978) that induces chromosomal aberrations and sister-chromatid exchanges (SCE) in root-tip cells of V. faba (Evans & Scott, 1964; Kihlman, 1975).

The present work was designed to study the cytotoxic effect of the maleic hydrazide (MH) herbicide using Trigonella foenum-graecum L. as a biological system.

Materials and Methods
Dry and healthy seeds of Trigonella foenum-graecum were pre-soaked in distilled water for 12 h, treated with Maleic Hydrazide (MH) at 6 different concentrations (0.01%-0.06%), and prepared in sodium phosphate buffer with 7.0 pH for 8 h with constant intermittent shaking. The treated seeds were washed in running tap water 2 to 3 times to remove the residual effect of the mutagen sticking to the seed coat. One set of the seeds was kept untreated to act as control for comparison. Hundred seeds were used from each dose and control. In order to find out the effect of MH on seed germination and root tip collection, 50 seeds were spread over moist cotton in petri plates and kept in a B.O.D. incubator at 24±2 °C temperature. Other sets of 50 seeds of control and 6 treatments of maleic hydrazide were sown separately in a randomized pot design with 3 replicates for the study of pollen fertility in control and treated populations in M, generation.

The root tips were excised and pre-treated with 0.02% Para-dichlorobenzene for 3 h, washed with distilled water, fixed in glacial acetic acid: ethanol (3:1) for 24 h, again washed with distilled water and stored in 70% alcohol. For cytological observations, the fixed roots were hydrolyzed at 60 °C for 15 min. in 1/10 N HCl and stained in Leuco Basic Fuchsin for 10-20 min. (Darlington and La Cour, 1976). The deeply stained region of root tips was squashed in 1% acetoarmarine. Photomicrographs were taken from both temporary as well as permanent slides.

Mitotic index (MI), relative division rate (RDR) and relative abnormality rate (RAR) were calculated by

\[
MI = \frac{\text{Total number of dividing cells}}{\text{Total number of cells examined}} \times 100
\]

\[
RDR = \frac{\text{Percentage of dividing cells in treated root tips}}{\text{Percentage of dividing cells in control root tips}} \times 100
\]

\[
RAR = \frac{\text{Percentage of dividing cells in root tips}}{\text{Percentage of dividing cells in control root tips}} \times 100
\]

Lethal Dose-50 (LD50)
LD50 (dose reducing germination up to 50%) was determined for maleic hydrazide on the basis of the total number of seed germinated in a treatment. Seeds giving rise to both radicle and plumule were considered as germinated. For each treatment and control, the percentage of seed germination was calculated by the following formula:

\[
\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds placed for germination}} \times 100
\]

After having calculated the germination percentage, LD50 dose was determined by the graph plotting the germination percentage along Y-axis against the concentration of the maleic hydrazide along X-axis, and the value of the LD50 dose was worked out with the help of the germination curve.

Statistical Analysis
The experimental data were statistically analyzed using the analysis of variance techniques according to Gomez and Gomez (1984). In applying the F test, the error due to replicates was also determined. When the F value was found to be significant at 1% and 5% level of probability, the least significant difference (LSD) was calculated.
Observations

Maximum germination was recorded in control (98%), whereas it decreased in treated populations from 92%-60% in (0.01%-0.06%) maleic hydrazide. The LD50 dose was 0.078% for maleic hydrazide treatments. Pollen fertility also decreased with the increasing concentrations of the mutagen. The mitotic index in control was observed to be maximum (19.30%), but decreased from 17.90%-5.84% in 0.01%-0.06% maleic hydrazide concentrations (Table 1). The relative divisional rate (RDR) following cytotoxic effect of mutagen came down from 1.74% to 16.68% in 0.01% to 0.06% maleic hydrazide. Consequently, the relative abnormality rate (RAR) out of the total number of cells increased as the concentrations of the mutagen increased but it did not show any linear dependence and the relative abnormality rate out of the total number of dividing cells increased significantly from 4.17% to 71.43% in 0.01%-0.06% MH (Table 1).

The behaviour of chromosomes at prophase and metaphase and their separation at anaphase and telophase was normal in control (Figure 1). As a result of MH treatment, multivalent formation at metaphase in 0.02% concentration (Figure 2), stickiness and precocious separation in 0.04% concentration (Figure 3), clumping of chromosomes at metaphase in 0.06% concentration (Figure 4), fragments in 0.03% concentration (Figure 5), and single & double bridges at anaphase in 0.04% and 0.05% concentrations (Figures 5 and 6, respectively) occurred in different frequencies and increased with increasing concentrations of mutagen (Figures 1-6).

Discussion

The reduced germination due to inhibitory effect of chemical mutagens as observed in *Trigonella foenum-graecum* L. has also been reported in same plant by Laxmi and Gupta (1983), Jain and Agarwal (1993), Siddiqui and Hasan (2003), Siddiqui et al. (2007) and Siddiqui et al. (2008), and also in *Vicia faba* L. by Agarwal and Ansari (2001).

Several other mutagenic agents and heavy metals have been shown to inhibit the seed germination (Seregin & Kozhevnikova, 2006). Several factors such as oxygen concentration, light, moisture level, and incubation temperature are known to have an influence on seed germination (Isabelle et al., 2000).

Griffiths and Johnson (1962) and Srivastava (1979) considered that reduction in germination percentage was due to weakening and disturbance of growth processes

<table>
<thead>
<tr>
<th>Treatments (%)</th>
<th>Seed germination (%)</th>
<th>Percentage Inhibition</th>
<th>Pollen fertility (%)</th>
<th>Relative Red. in Pollen fertility</th>
<th>Mitotic Index (%)</th>
<th>Relative Division Rate (RDR)</th>
<th>Relative abnormality rate (RAR) as compared to total number of cells observed</th>
<th>Relative abnormality rate (RAR) as compared to total number of dividing cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>98±2.45</td>
<td>–</td>
<td>99.2±2.48</td>
<td>–</td>
<td>19.30±0.51</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>0.01%</td>
<td>92±2.30</td>
<td>6.13**±0.15</td>
<td>97.4±2.44</td>
<td>1.82**±0.05</td>
<td>17.90±0.48</td>
<td>-1.74**±0.04</td>
<td>0.75**±0.02</td>
<td>4.17**±0.10</td>
</tr>
<tr>
<td>0.02%</td>
<td>80**±2.00</td>
<td>18.37**±0.46</td>
<td>91.7±2.92</td>
<td>7.56**±0.19</td>
<td>16.50**±0.45</td>
<td>-13.47**±0.34</td>
<td>1.73**±0.04</td>
<td>10.52**±0.26</td>
</tr>
<tr>
<td>0.03%</td>
<td>76**±1.90</td>
<td>22.45**±0.56</td>
<td>88.9±2.22</td>
<td>10.38**±0.26</td>
<td>13.30**±0.41</td>
<td>-7.44**±0.19</td>
<td>3.34**±0.08</td>
<td>25.00**±0.63</td>
</tr>
<tr>
<td>0.04%</td>
<td>74**±1.85</td>
<td>24.49**±0.61</td>
<td>82.7±2.07</td>
<td>16.64**±0.42</td>
<td>7.70**±0.33</td>
<td>-14.38**±0.36</td>
<td>5.17**±0.13</td>
<td>66.67**±1.67</td>
</tr>
<tr>
<td>0.05%</td>
<td>65**±1.63</td>
<td>33.67**±0.84</td>
<td>78.9±1.97</td>
<td>20.46**±0.51</td>
<td>6.70**±0.19</td>
<td>-15.65**±0.39</td>
<td>4.45**±0.11</td>
<td>66.67**±1.67</td>
</tr>
<tr>
<td>0.06%</td>
<td>60**±1.50</td>
<td>38.78**±0.97</td>
<td>71.1±1.78</td>
<td>28.32**±0.71</td>
<td>5.84**±0.17</td>
<td>-16.68**±0.42</td>
<td>4.16**±0.10</td>
<td>71.43**±1.79</td>
</tr>
<tr>
<td>LSD at 5% (*)</td>
<td>7.13</td>
<td>2.09</td>
<td>7.89</td>
<td>1.32</td>
<td>1.26</td>
<td>0.99</td>
<td>0.28</td>
<td>3.90</td>
</tr>
<tr>
<td>LSD at 1% (**)</td>
<td>8.77</td>
<td>2.57</td>
<td>9.71</td>
<td>1.62</td>
<td>1.54</td>
<td>1.22</td>
<td>0.35</td>
<td>4.80</td>
</tr>
</tbody>
</table>
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Figure 1. Root tip cell at normal anaphase with 8 chromosomes at each pole (control).

Figure 2. Root tip cell at metaphase showing 2-IV and 8-I formation (0.02%).

Figure 3. Root tip cell at metaphase showing stickiness and precocious separation of chromosomes (0.04%).

Figure 4. Root tip cell at metaphase showing clumping of chromosomes (0.06%).
resulting in early elimination of seedlings. The inhibition of growth regulators and metabolic disturbances during germination may also be one of the reasons (Sideris et al., 1971).

The pollen fertility decreased significantly as seen in mitotic index (MI) with the increasing concentrations of maleic hydrazide. Ghareeb and George (1997) suggested that Temik, an insecticide, may be found to cause an identical effect on mitosis and meiosis, increasing similar anomalies. Armbruster et al. (1991) also suggested that the induction of meiotic and mitotic abnormalities seems to be a common effect of most pesticides. In these experiments, it was observed that the chromosomal abnormalities in pollen mother cells (PMCs) increased proportionally to the increased mitotic aberrations. Hence the increased heterozygosity in PMCs resulted in decreasing pollen fertility. Chary and Bhalla (1988) and Kumar et al. (2003) also reported an increase in pollen sterility with an increase in mutagenic treatment. Rana and Swaminathan (1964) and Ramanna (1974) reported that any deviation in karyokinesis or cytokinesis could produce non-viable microspores. Sinha and Godward (1969, 1972) described translocations to be responsible for decreased pollen fertility.

The mitotic index is a reliable predictor of the cell proliferation in the tissue. Compared to control, a decrease in the mitotic index in root tip meristems of *Trigonella foenum-graecum* was observed in treated populations. The mitodepressive effect of the pesticide may be due to the interference of pesticide in the normal process of mitosis by reducing the number of the dividing cells (Habib et al., 1988). The inhibition in mitotic index (MI) may be due to the interference of Temik pesticide in the normal sequence of cell division, which prevented or reduced the number of the cells to enter prophase stage (Ghareeb & George, 1997). Concerning with the reduction of MI, several pesticides belonging to different chemical groups were found to cause an inhibition of cell division, such as Gespax (Badr et al., 1985), Sencor (Haliem, 1990), and Cycluron (Ghareeb, 1991).

As a result of mutagenic treatment of seed with maleic hydrazide, the root tip cells of *Trigonella foenum-graecum* showed varying degrees of mitotic irregularities like stickiness, univalents, multivalents, fragments, precocious separations of chromosomes, bridges with or without fragment which generally increased with the increasing concentrations of MH. Several mutagens with mutagenic activity have been shown to induce such changes in the...
chromosomes of plant cells (Amer & Ali, 1969, 1974). All the pesticidal treatments impaired spindle mechanisms to varying degrees leading to chromosome vagrancies as well as C-metaphase. Inhibition of ATPases by the pyrethroids might be the cause of spindle disorganization (Reddy & Bashamohideen, 1991). Precocious separation was also observed in tomato (Bose & Saha, 1970) and concluded that univalents separating precociously seemed to be as a result of desynapsis. According to Roy et al. (1971) precocious separation of bivalents in Cucumis sativus L. was attributed to the failure of chiasma formation in pairs.

Rao and Laxmi (1980) attributed univalent formation to the partial and complete lack of homologous chromosome pairing. According to Katiyar (1978) alteration in the chromosome associations, composed of uni-, tri-, tetra-, and multi-valent formations, were possibly the outcome of the non- or irregular pairing of chromosomes due to translocations.

The stickiness of chromosomes was the most common abnormality observed during the present investigation. Bivalents were found clumped into a single group due to stickiness. Similar results have also been reported by Abbasi and Anis (2002) in Trigonella foenum-graecum. Stickiness could be due to depolymerization of nucleic acid caused by mutagenic treatment. Stickiness in chromosomes is a result of partial dissociation and altered pattern of organization of nucleoprotein (Evans, 1962).

Presence of chromosome bridges may be due to stickiness or formation of dicentric chromosomes caused by breakage and reunion (Bempong, 1973). The stickiness of chromosomes makes their separation and free movement incomplete and thus they remain connected by bridges (Kabarity et al., 1974). This may also be due to the defective formation of the spindle apparatus (Badr, 1986; Abraham & Rajalakshmy, 1989).

Fragmentation might have arisen due to stickiness of chromosomes and consequent failure of separation of chromatids to poles and was also observed by many workers (Amer, 1965; Permjit & Grover, 1985 etc.). Fragments may also beacentric chromosomes that are formed as a result of inversion.

It may be concluded that MH causes toxic effect on root tip cells and other vegetative, as well as spore mother cells of Trigonella foenum-graecum. This toxicity induces different types of genic and chromosomal variations and enhances the rate of variations in morphological and yield characters for selection of better qualities and also increases the speed of evolution.

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