Gamma Ray Induced Cytomorphological Variations in *Sesbania cannabina* Poir.

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**Summary** Seeds of *Sesbania cannabina* cultivar ND-1 were subjected to different doses of gamma irradiation (20, 40, 60 and 80 KR) and irradiation effects on seed germination, survival, and several morphological and cytological characters of adult plants were studied. The irradiated seeds were tested for lethal doses at which 50% of the seeds germinated (LD$_{50}$) i.e. 40 KR dose. Plants grown from the gamma ray-treated seeds showed variation regarding their morphological and cytological characters. The plant height, stem girth, number of leaves per plant, and pod length decreased as the doses of irradiation increased. However, internode length increased with increasing doses of irradiation. Meiotic analysis revealed the presence of various types of chromosomal abnormalities at all phases. Meiotic aberrations increased according to the doses of irradiation. A reduction in pollen fertility was also noticed.

**Key words** LD$_{50}$, Gamma irradiation, Meiotic aberrations, Morphological parameters, *Sesbania cannabina*.

Mutation breeding is one of the most effective tools to induce variations in different qualitative and quantitative characters of crop species (Gregory 1955). Crop improvement using classical induced mutagenesis is now well standardized. A large number of new promising varieties in different crops have successfully been developed worldwide using both physical and chemical mutagens. As genetic variability is essential for crop improvement programmes, the creation and management of genetic variability have become central themes in crop breeding. X-ray, gamma ray, fast neutron, thermal neutron, ultraviolet and beta radiations are frequently used physical mutagens (Yaqoob and Rashid 2001). Gamma irradiation can bring about biochemical and biophysical changes that could affect the nutritional and functional properties of irradiated seeds (Dogbevi *et al.* 2000).

Over the past few decades, there has been a significant upsurge in the use of green manure for agriculture and considerable attention has been given to leguminous plants. *S. cannabina* is a quick growing, succulent, suffructicose, annual leguminous plant and ideally suited as a green manure crop. In India, the species has been introduced as green manure to enhance organic matter in rice fields. *Sesbania* green manuring has substantially improved grain yield, up to 72% (Bhatti *et al.* 1983). Because of its ability to grow in heavy metal soils and to withstand water logging and tolerate soil salinity, it is often the preferred green manure crop for rice and wheat (Ye *et al.* 2001). It yields a strong useful fibre, durable especially under water (Watt 1968). *S. cannabina* shows promises for early fodder and fuel production (Hossain *et al.* 2001).

The role of induced mutation in the development of novel variants for achieving genetic improvement in the species is yet to be explored. Therefore, our study is an attempt to collect, correlate and interpret the data for various categories of morphological variations and cytological aberrations induced by gamma rays in *S. cannabina*.

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Materials and methods

Seeds of *S. cannabina* cultivar ND-1 were obtained from the Sunn Hemp Research Station, Pratapgarh, U.P., India. Dry and healthy seeds were irradiated at 4 doses of gamma rays viz. 20, 40, 60 and 80 KR using $^{60}$CO source at the National Botanical Research Institute (N.B.R.I.), Lucknow. The dose rate was 18 Krad/s with gamma chamber-900 model (Bhabha Atomic Research Centre, Mumbai, India). After the irradiation, the seeds were sown in their respective pots in triplicate to raise the M1 generation. Along with the irradiated seeds, unirradiated seeds were also sown for a control set. Field experiments were carried out to determine the effects of gamma rays on germination, survival and on 5 different morphological parameters (plant height, stem girth, internodes length, and number of leaves per plant and pod length). Germination was taken after 15 d of sowing and survival percentages were taken after 30 d of sowing. All morphological data were taken after 45 d of sowing. On the onset of budding, buds were fixed in Carnoy’s fixative (1 : 3, glacial acetic acid : ethyl alcohol) in their respective bottles for 24 h and then stored in 70% ethyl alcohol in a refrigerator and were used for cytogenetic analysis using 2% acetocarmine. The frequency of meiotic aberration was counted in 200–400 pollen mother cells for each anther. Pollen fertility was evaluated by acetocarmine stainability test.

Results

**Effect of gamma rays on germination, survival and pollen fertility**

The control exhibited 90% germination and seeds irradiated at 20, 40, 60 and 80 KR doses exhibited germination at 60, 50, 40 and 30%, respectively (Table 1). The reduction in germination percentage was recorded as the doses of irradiation increased as compared to control. LD$_{50}$ in germination was 40 KR. Survival percentages also decreased depending on the dose of irradiation. The control and 20 KR sets exhibited 100% survival while this decreased further with increases in doses. Pollen fertility was 94.16% in the control as compared to the irradiated sets where it tended to be reduced in parallel with the increase in dose of irradiation; the lowest fertility rate recorded was 70% at 80 KR shown in Table 1.

**Table 1.** Germination, survival of seeds and pollen fertility of plants from irradiated seeds in *S. cannabina* at different doses of gamma rays irradiation.

<table>
<thead>
<tr>
<th>Irradiation dose (KR)</th>
<th>Germination (%)</th>
<th>Survival (%)</th>
<th>Pollen fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90</td>
<td>100</td>
<td>94.16</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
<td>100</td>
<td>81.66</td>
</tr>
<tr>
<td>40</td>
<td>50</td>
<td>90</td>
<td>80.33</td>
</tr>
<tr>
<td>60</td>
<td>40</td>
<td>75</td>
<td>74.66</td>
</tr>
<tr>
<td>80</td>
<td>30</td>
<td>25</td>
<td>70.00</td>
</tr>
</tbody>
</table>

*15 seeds were sown for each dose. **5 plants were observed at each dose for pollen fertility and 150–300 pollen grains/anther were observed.

**Table 2.** Mean performances of 5 morphological parameters.

<table>
<thead>
<tr>
<th>Irradiation dose (KR)</th>
<th>Plant height (cm)</th>
<th>Stem girth (cm)</th>
<th>Internode length (cm)</th>
<th>No. of leaves per plant</th>
<th>Pod length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90.25±5.63</td>
<td>2.42±0.20</td>
<td>4.55±0.22</td>
<td>18.50±1.55</td>
<td>22.47±0.37</td>
</tr>
<tr>
<td>20</td>
<td>91.00±8.86</td>
<td>2.17±0.08</td>
<td>4.95±0.29</td>
<td>18.00±1.29</td>
<td>14.65±0.13</td>
</tr>
<tr>
<td>40</td>
<td>88.00±4.26</td>
<td>1.92±0.07</td>
<td>5.38±0.14</td>
<td>17.25±1.25</td>
<td>13.97±0.16</td>
</tr>
<tr>
<td>60</td>
<td>87.75±6.06</td>
<td>1.85±0.06</td>
<td>5.51±0.13</td>
<td>16.25±0.62</td>
<td>9.67±0.08</td>
</tr>
<tr>
<td>80*</td>
<td>41.00±1.00</td>
<td>1.45±0.05</td>
<td>5.92±0.01</td>
<td>13.50±0.50</td>
<td>6.40±0.24</td>
</tr>
</tbody>
</table>

4 plants were measured in control plant and plants from the seeds irradiated of each dose. *Only 2 plants were survived.
Effect of gamma rays on morphological parameters

Table 2 shows the effect of gamma rays on 5 morphological parameters of adult plants from irradiated seeds. Plant height for different doses showed the clear-cut effect of irradiation. Among different doses of irradiation, maximum height was observed at 20 KR and minimum at 80 KR, i.e. 91.00±8.86 cm and 41.00±1.00 cm, respectively. The height of plants at 80 KR was found to be greatly reduced as compared to that of the control plants. Stem girth also reduced with the increase in irradiation dose. Maximum stem girth (2.42±0.20 cm) was recorded in the control followed by

2.17±0.08, 1.92±0.07, 1.85±0.06 and 1.45±0.05 cm at 20, 40, 60 and 80 KR, respectively. The positive effect of gamma rays was observed in internode length. Internodal length increased with the increase in irradiation dose and was found to be the highest at 5.92±0.01 cm in 80 KR while the control was at 4.55±0.22 cm. The number of leaves/plants showed a reduction with the increase in gamma ray dose as shown in Table 2. Pod length showed remarkable variation at different doses of irradiation. Control plants exhibited normal pod length of 22.47±0.37 cm whereas that of 80 KR was reduced to 6.4±0.24 cm at.

**Effect of gamma rays on microsporogenesis**

Meiosis was perfectly normal in control plants with 12 bivalents at metaphase I (Fig. 1) and 12:12 segregation at anaphase I (Fig. 2). However, the plants in gamma irradiated sets exhibited various types of aberrations at each phase of meiosis. Table 3 showed that different types of aberrations increased along with the increase in dose of gamma rays. Gamma rays induced various types of chromosomal aberrations such as univalents (Fig. 3), precocious movement (Fig. 4), multivalents, unorientation (Fig. 9), laggards, bridges (Fig. 5), stickiness, disturbed polarity etc. The most prominent abnormality was a laggard at anaphase I, unorientation and multivalent formation at metaphase I. Laggards (Fig. 7) were found to be the highest at 80 KR i.e. 2.67%. Bridges were observed at both anaphase I and II and telophase I and II but was at its highest in anaphases I and II. Univalents and multivalents (Fig. 8) at metaphase I were prominent at higher doses but found to be absent at 20 KR, whereas in metaphase II univalents and multivalents were absent at 80 KR. Scattering at metaphase I (Fig. 6) was observed only at the two lower doses and was absent at 60 KR and 80 KR.

**Discussion**

Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and mor-
phogenetic changes in cells and tissues (Moore and Hough 1962). The higher exposures are usually inhibitors of seed germination in angiosperms and gymnosperms (Dhakshanamoorthy et al. 2011, Akhaury and Singh 1993, Majeed and Muhammad 2010). The impact of gamma radiation on \textit{S. cannabina} and its tolerance levels to it manifested itself in terms of germination and survival percentages, which decreased with increasing doses; results similar to the present investigation were also reported by Tah (2006). In some plants, like cowpea, mungbean and Bengal gram, an LD50 value for gamma rays was observed between 20–25 KR, reported by Lois and Kadambavasundaram (1973), Palaniswamy (1975) and Vadivelu (1979), respectively. LD_{50}=40 KR for \textit{S. cannabina} in the present study is therefore higher than other bean species. Thus it can be concluded from the above that the gamma rays were less harmful to the germination process of \textit{S. cannabina} as compared to cowpea, mungbean and Bengal gram. A reduction in survival percentage due to gamma ray exposure was also reported in soybean (Pepol and Pepo 1989, Pavadai et al. 2009); mung bean (Khan and Wani 2005) and sesame (Prabhakar 1985).

The negative effect of gamma rays on pollen fertility may be due to the cumulative effect of various meiotic aberrations. Any disturbance in karyokinesis or cytokinesis could result in nonviable microspores (Ramanna 1974).

Retardation of growth process is one of the most common responses of plants subjected to ionizing radiation. Plant height was found to be significantly reduced at higher doses of irradiation but a slight stimulating effect was observed at the lowest dose of irradiation \textit{i.e.} some of the plants at 20 KR responded positively to mutagenic irradiation and showed a slight increase in plant height in comparison to control plants. The highest dose (80 KR) of gamma rays results in extreme dwarf plants. The adversely affected plant height was also reported by Shakoor \textit{et al.} (1978). The lowest dose furthermore offered maximum potential for increasing in plant height, which can be an economically useful character. Stem girth also displayed a negative relationship with irradiation.

A significant increase in internode length was observed in irradiated plants as compared to the control. In this aspect, a positive effect of gamma rays was observed. The increase in internode length was highest at 80 KR with respect to the control. Any increase in internode length is considered a valuable selection criterion for a plant which is an important source of fibre.

The induction of cytological disturbances in meiotic cells is of great value, as it results in genetic damage that is handed over to the next generation (Kumar and Rai 2007). Such induced chromosomal variations have been widely investigated from the point of view of understanding the mechanics of mutagen-induced chromosomal damage and biological dosimetry in \textit{S. cannabina}.

To deduce the most suitable dose to be used in a plant mutation breeding program, one has to understand the fact that plant lethality and chromosomal damage often increase with increasing doses. Hence, an intermediate dose has to be taken \textit{i.e.} between the low dose with lower mutation frequency but higher survival rate and the high dose with higher mutation frequency but lower survival rate. In the present investigation, LD_{50} was found at a 40 Kr dose of gamma rays. Hence, on the basis of the present cytogenetical study, we suggest that the most suitable dose of gamma rays for inducing mutation in \textit{S. cannabina} is 40 Kr. Furthermore, the evidence reviewed in the present investigation strongly suggests that the variations induced by the gamma rays provide a good scope for further improvement of this green manure crop.

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References


