In vitro mutagenesis in rose (Rosa hybrida L.) cv. Raktima for novel traits

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The efficiency of gamma-ray was worked out in generating mutant populations of Rosa hybrida L. cv. Raktima. Single node cuttings (25 cuttings per treatment with 3 replications) were irradiated with different doses of gamma-rays (0, 5, 10, 15, 25, 40, 55, 65, 70 & 80 Gy) using a $^{60}$Co source and then cultured aseptically on Murashige and Skoog basal medium supplemented with 13.31 µM 6-benzyl aminopurine (BAP) plus 0.54 µM α-napthalene acetic acid (NAA) plus 1.44 µM gibberellic acid (GA$_3$) plus 0.8% w/v agar-agar to induce sprouting and shoot proliferation. The 40 Gy-treatment was found to be the LD$_{50}$ dose. Morphological abnormalities, such as, fused leaves, leaf albinism and variegated leaves, were observed at the higher doses (25, 40 & 55 Gy). In vitro raised mutant plants and non-irradiated (control) plants were transferred to plastic pots 1 month after acclimatisation and examined for their morphological traits. Two types of flower colour mutants with altered or novel flower colour in comparison to original flower colour were isolated. The aim of the present study was to develop a protocol for induction of mutagenesis that could be used successfully to develop novel traits in rose.

Keywords: Gamma irradiation, LD$_{50}$, mutants, rose, single node cuttings

Introduction
Rose (Rosa hybrida L.; Family: Rosaceae) is an important ornamental crop, with seven chromosome numbers ($x$=n=7) and comprises of more than 150 species. It is valued in the international cut flower market because of its attractive flowers with longer vase-life$^1$. Besides cut flower, it is also used as loose flower, bush, climber, pot plant, potpourris and dry flowers. Demand and quest for novelty in roses is increasing day by day in the domestic as well as international flower industry. To meet the demand, it is essential to produce new varieties and scope for breeding roses for novel characters always exists. Apart from methods like selection, hybridization and natural mutation or isolation of natural bud sport, mutation-assisted breeding could be the potential methods to create novel variants in vegetatively propagated crops$^1$. Mutation breeding is an established method for crop improvement and has played a major role in the development of many new flower colour/shape mutants in ornamentals$^1$. Conventionally, in vegetatively propagated ornamentals, rooted cuttings are treated with gamma rays before planting. In these treated plants, mutation appears as chimeras, which remains the main bottleneck in mutation breeding$^2$. However, in vitro culture methods have facilitated the use of mutation-assisted breeding technique for improvement of both seeded and vegetative propagated crops$^3$. Induced mutation in combination with in vitro culture technique is now being considered as an effective method for plant improvement in several vegetative propagated crops because it provides an opportunity to increase variability of an economically important cultivar.

Sectorial somatic mutations in flower colour with mutated ray florets (yellow colour) were detected, when rooted cuttings of white flowered cv. ’Purnima’ and red flowered cv. ’Colchi Bahar’ of Chrysanthemum morifolium were treated with gamma rays. Ray florets were cultured in vitro and transferred to the field after rooting. These isolated yellow mutants flowered true-to-explant, floret colour, shape and have proved to be true-to-type in two successive generations$^4$. In vitro mutagenesis to induce novel mutants in rose has also been reported previously$^5$. The objective of the present study was to use an in vitro mutation technique to improve rose in order to select the desirable novel characters that can be utilized further in breeding programmes.

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Materials and Methods

Plant Material and Irradiation Treatment

Shoots from 2 to 3-yr-old, vigorous and healthy plant of rose cv. 'Raktima' were obtained from the field of Division of Floriculture and Landscaping, IARI, New Delhi, India and used as a source of in vitro explants. Single node cuttings were treated with different doses (5, 10, 15, 25, 40, 55, 70 & 80 Gy) of gamma rays using $^{60}$Co source at the Nuclear Research Laboratory, IARI, New Delhi. In vitro cultures were initiated following a protocol that was standardize before gamma ray treatment of explants and the cultures were maintained at 25±1°C under cool fluorescent lamp at 47 $\mu$mol m$^{-2}$ s$^{-1}$ with a 16 h photoperiod$^6$.

Culture Establishment, Shoot proliferation and LD$_{50}$ dose

After gamma irradiation, explants were washed with Teepol™ (detergent) (0.1%) solution for 5 min to remove dirt, followed by thorough washing under running tap water to remove residue of the detergent. The nodal segments were then pre-treated with the solution comprising carbendazim (0.2%) plus mancozeb-45 (0.2%) plus 8-hydroxy quinoline citrate (HQC) (200 ppm/L) to minimize the microbial contamination in cultures. Furthermore, the nodal explants were surface sterilized by immersing in 0.1% aqueous solution of mercuric chloride for 5 min, followed by three rinses with sterile distilled water. Surface sterilized explants were cultured aseptically on Murashige and Skoog medium supplemented with 13.31 $\mu$M 6-benzyl aminopurine (BAP) plus 0.54 $\mu$M $\alpha$-napthalene acetic acid (NAA) plus 1.44 $\mu$M gibberellic acid (GA$_3$) plus 0.8% w/v agar-agar (tissue culture grade; Qualigens, Mumbai) to establish the culture, then sub-cultured three times on the same medium for shoot proliferation. The LD$_{50}$ dose of gamma irradiation was estimated on the basis of number of explants survived and their in vitro growth.

Shoot Elongation

Shoots that multiplied on 13.31 $\mu$M BAP+0.54 $\mu$M NAA+1.44 $\mu$M GA$_3$+0.8% w/v agar-agar (Tissue Culture Grade; Qualigens, Mumbai) were separated and individual microshoots were transferred onto elongation medium consisting of basal Murashige and Skoog medium supplemented with 1.44 $\mu$M GA$_3$.

For rooting, elongated shoots were transferred individually to conical glass vessels (250 mL) containing $\frac{1}{2}$ Murashige Skoog basal medium supplemented with 2.69 $\mu$M NAA+ 2.46 $\mu$M BAP+ 40 g L$^{-1}$ sucrose+ 0.8% w/v agar-agar.

Acclimatization

In vitro rooted plantlets were carefully removed from conical glass vessels (250 mL) and washed thoroughly using autoclaved doubled-distilled water to remove all traces of 0.8% w/v agar-agar. The roots were then dipped in 0.1% carbendazim for 30 seconds and transferred to glass jars (500 mL), with polypropylene lids, filled with a 1:3 (v/v) vermiculite:agro-peat moistened with 25% of MS basal liquid medium for acclimatization.

Statistical Analysis

The data were analyzed based on simple completely randomized design (CRD) and the percent data were subjected to square root transformation before carrying out ANOVA.

Results and Discussion

In the present investigation, LD$_{50}$ values were determined at two stages, viz., sprouting and survival of nodal cuttings after gamma irradiation, in rose cv. Raktima. The 40 Gy treatment was found to be the LD$_{50}$ dose based on 50.38% explant survival as presented in Table 1 and Fig. 1. The explant survival and sprouting were found to be decreased linearly with increasing dose of gamma rays (Figs 1 & 2). The highest rate of explant survival (93.41%) was observed in unexposed control explants, whereas the minimum explant survival (12.17%) was observed at 80 Gy dose. Explants exposed to higher doses (65, 70 & 80 Gy) showed deleterious effect of ionizing radiations and a very few explants sprouted with poor growth that failed to survive after first sub-culture. The earliest bud sprouting (6.66 d) was recorded at 10 Gy, followed by 6.88 d at 5 Gy; whereas in control treatment, it was 7.24 d. The maximum delay in bud sprouting of 15.25 d was recorded in the 55 Gy treatment.

The reduction in explant survival was due to the harmful effects of ionising radiation and may be attributed to a decline in the levels of auxins and/or to increased chromosomal aberrations caused by gamma irradiation$^7$. Standardisation of optimal doses for ionizing radiation mutagens to plant tissue culture and their response on in vitro mutation efficiency has already been reported in rose and chrysanthemum$^8-9$.

The mutagenic treatments followed by tissue culture of nodal explants enhanced the number of
shoots regenerated from each explants in 5 and 10 Gy gamma ray dose over the untreated control plants. In general, rate of shoot multiplication gradually decreases with increase in gamma irradiation doses as presented in Table 1. The highest number of shoots (8.86) was formed after three sub-cultures at 5 Gy dose, whereas the minimum number of shoots (2.53) was observed at 55 Gy treatment. The maximum increase in shoot length (3.67 cm), inter-nodal length (1.60 cm) and number of leaves per plant (5.10) was observed in untreated (control) plants, followed by 5 Gy with shoot length (3.31 cm), inter-nodal length (1.57 cm) and number of leaves per plant (4.50). Shoot growth was markedly reduced at higher doses. In 55 Gy gamma ray dose shoot length (1.16 cm), inter-nodal length (0.82 cm) and number of leaves per plant (2.93) was found to be the minimum. Similar observations have been reported earlier in rose and gladiolus. Inhibitory effect of gamma rays on number of shoots has also been reported by several workers in rose and gladiolus.

In the present study, incidence of vegetative leaf abnormalities with respect to abnormal leaf shape, leaf lamina, fused leaves, albinism on leaves with less chlorophyll, variegated leaves with stunted growth etc. were noticed at higher doses of gamma ray in cultures derived from treated nodal explants with 25 Gy (12.44%), 40 Gy (13.63%) and 55 Gy (14.80%) doses of gamma irradiation (Table 1). Such abnormalities were detected in the early growth stages after irradiation treatment, which were usually

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**Table 1**—Effect of gamma irradiation on *in vitro* culture establishment, shoot proliferation and rhizogenesis in hybrid tea rose (*Rosa hybrida* L.) cv. Raktima

<table>
<thead>
<tr>
<th>Dose of gamma rays (Gy)</th>
<th>Explant survival (%)</th>
<th>Days to bud sprouting</th>
<th>No. of shoots per explant</th>
<th>Shoot length (cm)</th>
<th>Internodal length (cm)</th>
<th>No. of leaves per shoot</th>
<th>Leaf abnormalities (%)</th>
<th>Days to root initiation</th>
<th>Rooting (%)</th>
<th>No. of roots per shoot</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 93.41 (75.22)</td>
<td>7.24 6.51 3.67 1.60</td>
<td>5.10 0.00 (0.00)</td>
<td>11.20 94.30 (76.26)</td>
<td>6.57 5.63</td>
<td>19.10 86.23 (68.23)</td>
<td>4.10 0.00 (0.00)</td>
<td>15.63 87.33 (69.20)</td>
<td>4.33 3.70</td>
<td>5.10</td>
<td>0.73 0.29 0.40 0.40 0.52 1.58 0.29 2.40 0.30 0.36</td>
<td></td>
</tr>
<tr>
<td>5 89.63 (71.42)</td>
<td>6.88 8.86 3.31 1.57</td>
<td>4.50 0.00 (0.00)</td>
<td>11.56 93.40 (75.16)</td>
<td>6.55 5.39</td>
<td>19.10 86.23 (68.23)</td>
<td>4.10 0.00 (0.00)</td>
<td>15.63 87.33 (69.20)</td>
<td>4.33 3.70</td>
<td>5.10</td>
<td>0.73 0.29 0.40 0.40 0.52 1.58 0.29 2.40 0.30 0.36</td>
<td></td>
</tr>
<tr>
<td>10 87.54 (69.38)</td>
<td>6.66 8.20 2.33 1.17</td>
<td>4.26 0.00 (0.00)</td>
<td>13.53 90.30 (71.90)</td>
<td>5.96 4.43</td>
<td>19.10 86.23 (68.23)</td>
<td>4.10 0.00 (0.00)</td>
<td>15.63 87.33 (69.20)</td>
<td>4.33 3.70</td>
<td>5.10</td>
<td>0.73 0.29 0.40 0.40 0.52 1.58 0.29 2.40 0.30 0.36</td>
<td></td>
</tr>
<tr>
<td>15 75.28 (60.27)</td>
<td>9.28 5.60 2.23 0.77</td>
<td>4.10 0.00 (0.00)</td>
<td>14.44 (20.62)</td>
<td>19.10 86.23 (68.23)</td>
<td>3.73 2.76</td>
<td>5.10 0.00 (0.00)</td>
<td>15.63 87.33 (69.20)</td>
<td>4.33 3.70</td>
<td>5.10</td>
<td>0.73 0.29 0.40 0.40 0.52 1.58 0.29 2.40 0.30 0.36</td>
<td></td>
</tr>
<tr>
<td>25 64.66 (53.58)</td>
<td>11.95 4.46 1.70 1.03</td>
<td>3.80 12.44 (20.62)</td>
<td>22.30 85.07 (67.32)</td>
<td>3.73 2.76</td>
<td>19.10 86.23 (68.23)</td>
<td>4.10 0.00 (0.00)</td>
<td>15.63 87.33 (69.20)</td>
<td>4.33 3.70</td>
<td>5.10</td>
<td>0.73 0.29 0.40 0.40 0.52 1.58 0.29 2.40 0.30 0.36</td>
<td></td>
</tr>
<tr>
<td>40 50.38 (45.24)</td>
<td>12.57 3.57 1.56 0.98</td>
<td>3.30 13.63 (21.67)</td>
<td>22.30 85.07 (67.32)</td>
<td>3.73 2.76</td>
<td>19.10 86.23 (68.23)</td>
<td>4.10 0.00 (0.00)</td>
<td>15.63 87.33 (69.20)</td>
<td>4.33 3.70</td>
<td>5.10</td>
<td>0.73 0.29 0.40 0.40 0.52 1.58 0.29 2.40 0.30 0.36</td>
<td></td>
</tr>
<tr>
<td>55 30.03 (33.24)</td>
<td>15.25 2.53 1.16 0.82</td>
<td>2.93 14.80 (22.64)</td>
<td>25.60 76.10 (60.78)</td>
<td>3.73 2.50</td>
<td>19.10 86.23 (68.23)</td>
<td>4.10 0.00 (0.00)</td>
<td>15.63 87.33 (69.20)</td>
<td>4.33 3.70</td>
<td>5.10</td>
<td>0.73 0.29 0.40 0.40 0.52 1.58 0.29 2.40 0.30 0.36</td>
<td></td>
</tr>
</tbody>
</table>

* Arc Sin √% transformed data

Fig. 1—Survival percentages showing the LDso value (40 Gy) of irradiated nodal explants of rose cv. Raktima following different doses of gamma rays.

Fig. 2 (A-J)—Effect of different doses of gamma rays on the sprouting of nodal explants of rose cv. Raktima. [(A) 0, (B) 5, (C) 10, (D) 15, (E) 25, (F) 40, (G) 55, (H) 65, (I) 70, & (J) 80 Gy]
recoverable later on. However, plants were normal in control and in lower doses (5, 10 and 15 Gy) of gamma rays (Fig. 3).

The development of abnormal leaves in the gamma rays treated populations were probably due to physiological disturbances and chromosomal aberrations. The colour variation in leaves might be due to the non-availability of nutrients affecting of biochemical pathways. Abnormalities in colour form and texture of leaves as consequence of irradiation has been reported in rose, carnation and other ornamental crops by several workers.

Untreated shoots showed better rooting ability giving rise to the highest rooting (94.30 %) and mean number of roots per shoot (6.57) within shortest time period (11.20 d) with maximum mean root length (5.63 cm) as shown in Table 1. The maximum delay in rooting (25.60 d) with minimum percentage of rooting (76.10) and least number of roots per shoot (3.73) with shortest roots (2.50 cm) were recorded in 55 Gy gamma ray treatment. All the treatments showed significant differences among each other.

Thus *ex vivo* survival and morphological studies showed a significant reduction in the survival percentage, plant height, number of branches, number of leaves per plant, and inter-nodal length of plantlets at higher dose of gamma rays even after acclimatization (Table 2). The highest plant survival (93.20%), plant height (16.30 cm), number of branches (4.09) and number of leaves per plant (40.26) and inter-nodal length (3.24 cm) was registered in untreated control; whereas the minimum survival (61.33%), plant height (8.30 cm), number of branches (1.66), number of leaves per plant (14.70) and inter-nodal length (1.90 cm) was observed in 55 Gy of gamma rays.

The gamma irradiation and harsh external environment with variable temperature, low humidity and high light intensity might have contributed to the reduction in survival percentage and other growth parameters. Similar trend of reduction in plant height and other vegetative parameters in chrysanthemum and rose due to gamma irradiation was also reported earlier. The reduced vegetative growth parameters may be due to chromosomal damage and mitotic inhibition. The genetic injury causes differential killing of meristematic cells, as a result damaged cells produce less cell progenies. While mitotic inhibition induces physiological changes, inhibition of auxin synthesis leading to reduced growth, and inability of irradiated cells to absorb/utilize the available nutrients.

Effect of gamma irradiation on leaf colour and leaf shape was also observed. Leaves were found normal with a dark green colour in control (untreated plants) and at lower doses of gamma rays (5, 10 or 15 Gy), while light green-coloured leaves were observed at higher gamma-ray doses (25, 40 or 55 Gy). Morphological abnormalities in the foliage were induced at exposures to higher gamma ray doses, which include change in leaf shape and size, serrated margins and synthesis of less chlorophyll content (Table 2; Fig. 4). The maximum leaf abnormalities (8.35 %) was recorded in population raised in 55 Gy treatment. Whereas plants derived from 0, 5, 10 or 15 Gy treated nodal explants were phenotypically normal. Further, the effect of gamma irradiation on spine density was not so pronounced. The highest number (8.95) of spines per 5 cm of stem was...
recorded in the plants obtained from non-irradiated explant (control), followed by 5 Gy (8.22 spines per 5 cm) and 10 Gy (7.88 spines per 5 cm) gamma ray doses. However, slightly reduced prickle density per 5 cm of stem was recorded in the cv. Raktima at 40 Gy (7.63) and at 55 Gy (7.77) (Table 2).

Flower production in roses is an important character from commercial point of view. Flowering was significantly delayed in plants derived from nodal explants irradiated at higher doses of gamma ray. The maximum delay (63.01 d) in flowering was observed in 55 Gy treatment. However, flowering was markedly advanced at doses 5 Gy (43.27 d) and 10 Gy (43.40 d) (Table 2). Further, the highest number (3.60) of flowers per plant was recorded in control, followed by 5 Gy (3.50) and 10 Gy (3.33) gamma ray treatments. At 55 Gy dose, the production of flowers reduced to 1.50 flowers per plant. Gamma irradiation at lower doses showed more number of petals, length of flower bud and flower stalk length as compared to higher gamma ray doses as presented in Table 2. Number of petals, length of flower bud and flower stalk length were significantly influenced by different gamma ray treatments. The maximum number of petals (26.66), length of flower bud (1.86 cm) and flower stalk length (19.94 cm) was observed with control, whereas the minimum number of petals (10.26), length of flower bud (1.23 cm) and flower stalk length (12.94 cm) was recorded with 55 Gy treatment. All flowering characters differed in plants irradiated with different doses of gamma rays. The flower diameter decreased with increase in exposure to gamma rays (Table 2). Plants derived from control explants showed the maximum flower diameter.

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**Table 2—Effect of gamma irradiation on ex vitro survival and growth and floral parameters in hybrid tea rose** (*Rosa hybrida* L.) cv. Raktima

<table>
<thead>
<tr>
<th>Dose of gamma rays (Gy)</th>
<th>Survival (%)</th>
<th>Plant height (cm)</th>
<th>Internodal length (cm)</th>
<th>No. of branches per plant</th>
<th>No. of leaves per plant</th>
<th>Leaf variations (%)</th>
<th>Spine density (per 5 cm)</th>
<th>Days to flowering</th>
<th>No. of flowers per plant</th>
<th>No. of petals per flower</th>
<th>Length of flower bud (cm)</th>
<th>Length of flower stalk (cm)</th>
<th>Flower diameter (cm)</th>
<th>Flower colour variations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>93.20 (74.88)</td>
<td>16.30</td>
<td>3.24</td>
<td>4.09</td>
<td>40.26</td>
<td>0.00 (0.00)</td>
<td>8.95</td>
<td>44.42</td>
<td>3.60</td>
<td>26.66</td>
<td>1.86</td>
<td>19.94</td>
<td>8.53</td>
<td>0.0 (0.00)</td>
</tr>
<tr>
<td>5</td>
<td>92.50 (74.1)</td>
<td>14.00</td>
<td>3.15</td>
<td>4.03</td>
<td>34.87</td>
<td>0.00 (0.00)</td>
<td>8.22</td>
<td>43.27</td>
<td>3.50</td>
<td>25.36</td>
<td>1.86</td>
<td>19.82</td>
<td>8.50</td>
<td>0.0 (0.00)</td>
</tr>
<tr>
<td>10</td>
<td>86.43 (68.36)</td>
<td>12.46</td>
<td>3.14</td>
<td>3.36</td>
<td>32.20</td>
<td>0.00 (0.00)</td>
<td>7.88</td>
<td>43.40</td>
<td>3.33</td>
<td>22.60</td>
<td>1.73</td>
<td>19.27</td>
<td>8.16</td>
<td>0.0 (0.00)</td>
</tr>
<tr>
<td>15</td>
<td>77.06 (61.34)</td>
<td>11.16</td>
<td>3.13</td>
<td>3.30</td>
<td>24.60</td>
<td>0.00 (0.00)</td>
<td>8.17</td>
<td>46.80</td>
<td>2.73</td>
<td>21.60</td>
<td>1.53</td>
<td>18.34</td>
<td>7.46</td>
<td>0.0 (0.00)</td>
</tr>
<tr>
<td>25</td>
<td>71.96 (57.99)</td>
<td>11.03</td>
<td>3.11</td>
<td>2.50</td>
<td>20.73</td>
<td>7.36 (15.68)</td>
<td>7.91</td>
<td>52.62</td>
<td>2.50</td>
<td>15.76</td>
<td>1.36</td>
<td>16.68</td>
<td>7.43</td>
<td>3.92 (11.39)</td>
</tr>
<tr>
<td>40</td>
<td>71.06 (57.42)</td>
<td>9.90</td>
<td>2.01</td>
<td>1.80</td>
<td>18.66</td>
<td>8.00 (16.43)</td>
<td>7.63</td>
<td>54.93</td>
<td>1.63</td>
<td>10.96</td>
<td>1.33</td>
<td>12.33</td>
<td>7.20</td>
<td>7.46 (15.79)</td>
</tr>
<tr>
<td>55</td>
<td>61.33 (51.53)</td>
<td>8.30</td>
<td>1.90</td>
<td>1.66</td>
<td>14.70</td>
<td>8.35 (16.74)</td>
<td>7.77</td>
<td>63.01</td>
<td>1.50</td>
<td>10.26</td>
<td>1.23</td>
<td>12.94</td>
<td>7.16</td>
<td>8.51 (16.88)</td>
</tr>
<tr>
<td>(P≤0.05)</td>
<td>5.16</td>
<td>0.35</td>
<td>0.29</td>
<td>0.28</td>
<td>0.77</td>
<td>0.15 (0.30)</td>
<td>0.27</td>
<td>3.62</td>
<td>0.20</td>
<td>0.95</td>
<td>0.21</td>
<td>0.13 (0.13)</td>
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<td></td>
</tr>
</tbody>
</table>

* Arc Sin √% transformed data
(8.53 cm), while the minimum flower diameter (7.16 cm) was observed at 55 Gy gamma ray dose.

The delay in flowering in various ornamental crops following irradiation has also been reported in rose, chrysanthemum and gladiolus\textsuperscript{10}. The reduction in flower growth parameters at higher doses of gamma rays may be due to consequence of somatic competition, which takes place in the course of intra-somatic selection. Earlier, several workers have reported the effect of gamma rays on various crops such as iris, gladiolus, carnation and rose\textsuperscript{8,13,19}.

Plantlets without any variation in floral traits were observed in untreated control and at lower irradiation doses (5, 10 or 15 Gy). The treated nodal explants exhibited maximum flower colour variation (8.51\%) in 55 Gy treatments, while frequency of variation was 3.92\% and 7.46\% in 25 Gy and 40 Gy, respectively.

Two flower colour variants were isolated and multiplied through tissue culture. The details of both the variants are given below:

**RK-1 (Red with Stripes Variant):** This variant is having red coloured flower buds (red purple 58-C) with stripes (yellow orange 19-C) as compared to red in original cv. Raktima (control) derived at 40 Gy dose of gamma rays with mutation frequency of 7.46\%. Flowers were of medium size, with unfurled outer petals and compact from the center. Flower sepals in variant were straight with serrated margins as compared to curved and non-serrated margins (Fig. 5).

**RK-2 (Dark Red with Streaks Variant):** This variant was developed by 55 Gy gamma irradiation dose with mutation frequency of 8.51\%. Flowers were fully opened large having dark coloured petals (red 50-B) with streaks (white 158-D) as compared to red in original cv. Raktima (control). There were little differences in vegetative characters between variant and original cultivar such as four sepals in variants as compared to original cultivar (Fig. 5).

Thus protocol for \textit{in vitro} mutagenesis in rose cv. Raktima was established using physical mutagens (gamma ray) that can be used further for breeding programme. The present investigation reveals that irradiation of nodal explants with 40 and 55 Gy doses of gamma rays was found to be the effective doses for successful induction of novel variants. Induced mutations through tissue culture technique is highly desirable for developing new cultivars for floriculture industry and cut flower trade and to fulfill the demand of domestic and international market.

\begin{acknowledgement}

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\end{acknowledgement}

\begin{references}

\textsuperscript{1} Datta S K, \textit{Ornamental plants: Role of mutation} (Daya Publishing House, New Delhi) 1997.

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