Chromosomal Alterations in Mitotic and Meiotic System as Influenced by Gamma Rays in *Pisum*¹

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Radiation induced chromosomal alterations are widely known in the crop plants. In barley (Caldecott and Smith 1952), tomato (Yagyu and Morris 1957), *Tradescantia* (Sas 1941) and Jute (Basu 1962) several structural changes were reported through radiation treatment. The present text reports the study of alterations in mitotic and meiotic system as influenced by gamma rays in *Pisum*.

Materials and methods

Dry seeds of pea varieties T 163 and Duke of Albany were irradiated with three doses of gamma rays 10 kr, 20 kr, and 40 kr. Seeds were germinated in petri dishes lined with moist filter paper for collection of root tips when the roots were 4–5 cm long. Seeds from each dose of irradiation were space planted in the field.

*Pollen sterility and sporocytes collection:* Soon after initiation of flowering two to three buds from each tagged plants were collected in 70 per cent alcohol. One per cent acetocarmine was used for the study of pollen sterility. Pollen sterility was considered a suitable basis for selection of sporocytes for cytological studies.

Sporocytes were collected in 1:3 acetic alcohol from 8 to 10.30 A.M. in the morning for 24 hours. They were then stored in 70 per cent alcohol in a refrigerator. Haematoxylin stain was used for the study of root tip mitosis. Tips were hydrolyzed in N. HCl for approximately five minutes and stained with 1 per cent haematoxylin stain after passing the material in ferric ammonium sulphate mordant.

For meiotic preparations anthers were smeared on a clean slide in a drop of 1.5 per cent freshly prepared acetocarmine stain. Slight heating and pressing improved staining and separation of chromosomes.

Experimental findings

*Change in the mitotic system*

Assessment of the effect of gamma irradiation on chromosomes during mitosis was made from detailed study of metaphase and subsequent stages. The data

¹ This work was conducted in the Department of Genetics and Plant Breeding, Faculty of Agriculture, Banaras Hindu University, Varanasi-5, INDIA.
Figs. 1-9. 1a, normal metaphase. Showing 14 chromosomes. 1b. non-orientation. 2, breakage. 3, chromatid bridge. 4, chromosome bridge. 5, bridge with fragment. 6, lagging fragments (chromosomes). 7, lagged paired fragment. 8, irregular grouping of anaphase chromosomes. 9, micronucleus.
Table 1. Type and frequencies of aberrations at different stages of mitosis

<table>
<thead>
<tr>
<th>Variety and Dose</th>
<th>Metaphase</th>
<th></th>
<th>Anaphase</th>
<th></th>
<th></th>
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<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>Non-orientation</td>
<td>Fragment</td>
<td>Total</td>
<td>Single bridge</td>
<td>Double bridges</td>
<td>Bridges with fragment</td>
<td>lagging fragment</td>
</tr>
<tr>
<td>T 163</td>
<td>1.20</td>
<td>1.18</td>
<td>2.38</td>
<td>0.97</td>
<td>1.68</td>
<td>1.40</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>2.13</td>
<td>0.97</td>
<td>3.10</td>
<td>0.71</td>
<td>3.10</td>
<td>2.50</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>3.40</td>
<td>2.31</td>
<td>5.71</td>
<td>1.80</td>
<td>9.51</td>
<td>6.34</td>
<td>2.54</td>
</tr>
<tr>
<td>Duke of Albany</td>
<td>1.05</td>
<td>0.47</td>
<td>1.52</td>
<td>1.05</td>
<td>2.10</td>
<td>1.03</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>1.63</td>
<td>1.14</td>
<td>2.77</td>
<td>1.01</td>
<td>5.46</td>
<td>2.03</td>
<td>2.38</td>
</tr>
<tr>
<td></td>
<td>0.53</td>
<td>2.65</td>
<td>3.18</td>
<td>2.67</td>
<td>9.50</td>
<td>7.20</td>
<td>1.20</td>
</tr>
</tbody>
</table>
Figs. 10–16. 10, ring of four. 11, chain of four. 12, four univalents. 13, lagging chromosomes. 14, bridges with fragment. 15, irregular movement of chromosomes. 16, 6:8 separation at anaphase I.
1972 Chromosomal Alterations in Mitotic and Meiotic System as Influenced

are presented in Table 1. Analysis of cells at prophase was not made since chromosomes at prophase could not be precisely scored for aberrations.

The common chromosomal aberrations at metaphase were non-orientation and breakage (Figs. 1 and 2). The frequency of these abnormalities is presented in Table 1. Maximum frequency of non-orientation and fragments was 3.40 and 2.65 per cent respectively at 40 kr dose.

Chromosome and chromatid bridges at anaphase were considered as major criteria to measure the effect of radiation. At anaphase single bridge (chromatid bridge) (Fig. 3), double bridge (chromosome bridge) (Fig. 4), bridge with fragment (Fig. 5) and lagging fragments (Fig. 6) were observed. Some of the fragments occurred in pair (Fig. 7). Another feature is the irregular grouping of anaphase chromosome (Fig. 8). There is a wide range of variation in the frequency of these changes. Double bridges at anaphase were observed with the highest frequency (Table 1). A characteristic feature is the increase in the frequency of chromosomal changes with increase in the dose of radiation at metaphase and anaphase (Table 1). 40 Kr gave the maximum frequency of disturbances. Maximum frequency of changes was observed at anaphase stage. At telophase micronuclei were commonly observed (Fig. 9).

Change in the meiotic system

Some M1 plants selected on the basis of pollen sterility were analysed cytologically and their total aberration frequency was recorded (Table 2). Maximum frequency was exhibited at 40 Kr dose. Various types of chromosomal alterations were observed at different stages. Ring of four (Fig. 10), chain for four (Fig. 11) and varying number of univalents (Fig. 12) were observed at metaphase I. Frequency and other behaviour of translocations are published elsewhere. Lagging chromosomes (Fig. 13), bridges with fragment (Fig. 14) and irregular movement of chromosomes (Fig. 15) were common aberrations at anaphase I.

Types and frequency of different chromosomal aberrations at metaphase I are given in Table 3.

6II + 2I Configuration

This type of configuration was present in plants A1-6, A1-18, A3-24, A3-5, A3-58, A3-2, A3-39, A3-53, C1-8, C1-9, C1-28, C2-5, C2-11, C2-55 etc. with variable frequency (Table 3).

5II + 4I Configuration

An interesting observation in certain other plants was the high frequency of univalents either due to non pairing of chromosomes or complete terminalization of chiasmata. Eighteen such plants were observed which are listed in Table 3. Seven plants showed 6II+1 rod bivalents (Table 3).

Bridge with fragment

Other chromosomal abnormalities were observed at anaphase I. A bridge with fragment was present in three plants A1-18, A3-35 and C2-3 showing 10.5,
Table 2. Frequency of pollen and ovule sterility and total meiotic chromosomal aberrations in some M1 plants

<table>
<thead>
<tr>
<th>Variety and dose</th>
<th>M1 culture and plant number</th>
<th>Pollen sterility %</th>
<th>Ovule sterility %</th>
<th>Aberrant PMCs %</th>
<th>Total aberration per dose %</th>
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<tbody>
<tr>
<td><strong>T 163</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Kr</td>
<td>A 1-6</td>
<td>18.5</td>
<td>15.2</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 1-13</td>
<td>76.5</td>
<td>25.2</td>
<td>45.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 1-18</td>
<td>31.2</td>
<td>15.9</td>
<td>6.8</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>A 1-24</td>
<td>35.6</td>
<td>14.2</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 1-52</td>
<td>31.2</td>
<td>8.5</td>
<td>25.1</td>
<td></td>
</tr>
<tr>
<td>20 Kr</td>
<td>A 2-2</td>
<td>31.2</td>
<td>9.2</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 2-5</td>
<td>18.5</td>
<td>16.8</td>
<td>44.9</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>A 2-7</td>
<td>31.2</td>
<td>7.2</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 2-18</td>
<td>61.2</td>
<td>28.2</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 2-38</td>
<td>55.2</td>
<td>15.8</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 2-58</td>
<td>39.5</td>
<td>13.2</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>40 Kr</td>
<td>A 3-6</td>
<td>51.2</td>
<td>18.2</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 3-7</td>
<td>68.5</td>
<td>31.2</td>
<td>72.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 3-17</td>
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<td>15.2</td>
<td>10.2</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>A 3-39</td>
<td>66.2</td>
<td>28.5</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 3-46</td>
<td>15.2</td>
<td>10.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 3-53</td>
<td>38.5</td>
<td>19.2</td>
<td>7.2</td>
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<tr>
<td></td>
<td>A 3-59</td>
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<td>23.6</td>
<td>26.2</td>
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<tr>
<td><strong>Duke of Albany</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Kr</td>
<td>C 1-2</td>
<td>33.5</td>
<td>16.5</td>
<td>20.5</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>13.5</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 1-9</td>
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<td>18.5</td>
<td>15.6</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>C 1-28</td>
<td>45.4</td>
<td>16.8</td>
<td>39.6</td>
<td></td>
</tr>
<tr>
<td>20 Kr</td>
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<td>15.2</td>
<td>45.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 2-5</td>
<td>60.2</td>
<td>25.6</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 2-9</td>
<td>32.5</td>
<td>12.6</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 2-11</td>
<td>20.3</td>
<td>16.5</td>
<td>12.0</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>C 2-26</td>
<td>16.5</td>
<td>13.2</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 2-35</td>
<td>38.5</td>
<td>21.5</td>
<td>43.0</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>76.5</td>
<td>23.5</td>
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</tr>
<tr>
<td></td>
<td>C 3-6</td>
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<td>25.2</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 3-18</td>
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<td>16.3</td>
<td>10.8</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>C 3-19</td>
<td>27.5</td>
<td>13.8</td>
<td>72.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 3-34</td>
<td>18.9</td>
<td>18.9</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

38.5 and 15.4 per cent frequencies (Table 4).

**Lagging chromosomes**

During anaphase some chromosomes do not reach the pole but lag behind at the centre. Frequency of different plants showing such aberrations is presented
Table 3. Chromosomal aberrations in some partially sterile M₁ plants at metaphase I

<table>
<thead>
<tr>
<th>Culture and plant number</th>
<th>No. of cells studied</th>
<th>Configurations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7₁₁   1₈₄+5₁₁ Chain of four+5₁₁ 6₁₁+2₁ 5₁₁+4₁ 6₁₁+2 rod bivalents</td>
</tr>
<tr>
<td>A 1–6</td>
<td>506</td>
<td>94.6  —  —  33.7  1.5  —</td>
</tr>
<tr>
<td>A 1–13</td>
<td>316</td>
<td>54.7  *  *  —  0.6  —</td>
</tr>
<tr>
<td>A 1–18</td>
<td>402</td>
<td>94.0  —  —  5.6  1.2  —</td>
</tr>
<tr>
<td>A 1–24</td>
<td>302</td>
<td>96.5  —  —  2.5  0.8  —</td>
</tr>
<tr>
<td>A 1–52</td>
<td>185</td>
<td>75.0  *  *  —  —  —</td>
</tr>
<tr>
<td>A 2–2</td>
<td>310</td>
<td>83.5  —  —  —  —  —</td>
</tr>
<tr>
<td>A 2–5</td>
<td>602</td>
<td>55.0  *  *  1.2  —  —</td>
</tr>
<tr>
<td>A 2–7</td>
<td>215</td>
<td>97.2  —  —  —  —  —</td>
</tr>
<tr>
<td>A 2–18</td>
<td>184</td>
<td>91.0  *  —  —  —  —</td>
</tr>
<tr>
<td>A 2–38</td>
<td>215</td>
<td>98.5  —  —  —  —  —</td>
</tr>
<tr>
<td>A 2–58</td>
<td>195</td>
<td>91.4  —  —  2.3  6.3  —</td>
</tr>
<tr>
<td>A 3–2</td>
<td>250</td>
<td>86.5  —  —  2.5  10.8 —</td>
</tr>
<tr>
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<td>300</td>
<td>28.0  *  *  —  —  —</td>
</tr>
<tr>
<td>A 3–17</td>
<td>185</td>
<td>90.0  *  *  —  —  —</td>
</tr>
<tr>
<td>A 3–39</td>
<td>400</td>
<td>93.6  —  —  3.8  2.5  —</td>
</tr>
<tr>
<td>A 3–46</td>
<td>250</td>
<td>97.5  —  —  —  —  2.5</td>
</tr>
<tr>
<td>A 3–53</td>
<td>182</td>
<td>93.3  —  —  3.8  2.4  1.5</td>
</tr>
<tr>
<td>A 3–59</td>
<td>260</td>
<td>73.5  *  *  —  —  —</td>
</tr>
<tr>
<td>C 1–2</td>
<td>295</td>
<td>79.5  *  *  —  —  —</td>
</tr>
<tr>
<td>C 1–8</td>
<td>168</td>
<td>92.0  —  —  5.7  2.1  —</td>
</tr>
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</tr>
<tr>
<td>C 1–28</td>
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</tr>
<tr>
<td>C 2–3</td>
<td>250</td>
<td>54.0  *  *  2.8  —  —</td>
</tr>
<tr>
<td>C 2–5</td>
<td>300</td>
<td>85.6  —  —  5.6  8.5  —</td>
</tr>
<tr>
<td>C 2–9</td>
<td>152</td>
<td>57.0  —  —  5.6  2.9  —</td>
</tr>
<tr>
<td>C 2–11</td>
<td>165</td>
<td>87.8  —  —  5.5  6.5  —</td>
</tr>
<tr>
<td>C 2–26</td>
<td>194</td>
<td>99.0  —  —  —  —  0.8</td>
</tr>
<tr>
<td>C 2–35</td>
<td>280</td>
<td>57.0  *  —  —  —  —</td>
</tr>
<tr>
<td>C 2–55</td>
<td>155</td>
<td>10.2  —  —  55.2 34.5 —</td>
</tr>
<tr>
<td>C 3–5</td>
<td>290</td>
<td>46.2  *  *  2.3  9.4  —</td>
</tr>
<tr>
<td>C 3–6</td>
<td>185</td>
<td>92.0  —  —  4.8  3.3  —</td>
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<td>C 3–18</td>
<td>135</td>
<td>89.2  —  —  2.5  8.2  —</td>
</tr>
<tr>
<td>C 3–19</td>
<td>136</td>
<td>38.0  *  —  —  —  —</td>
</tr>
<tr>
<td>C 3–34</td>
<td>356</td>
<td>99.2  —  —  —  —  0.2</td>
</tr>
</tbody>
</table>

* Showing interchange frequency which are not mentioned here.

In Table 4. Plants A 2–35, C 1–28 and C 2–3 showed 6–1–7 type of separation while plants A 1–18, A 3–46, C 1–28 and C 2–3 showed 6–3–5 segregation. Another interesting observation was that 46.8 per cent disturbances were found at anaphase cells in plant A 3–46. 6–8 separation of chromosomes in some of the plants leads to the production of trisomic gametes (Fig. 16).
Chromosomal alterations may be resulted by chemical changes during breakage and reunion of chromosomes which may be related to the metabolic state of the cell and dose of radiation. One of the causes of disturbances may be the inhibition of onset of DNA synthesis in the nucleus through ionizing radiation.

Chromosome breakage through radiation was reported by Wolff and Luippold (1956). Presence of paired fragment at anaphase in the present observation indicate that the chromosome behaved as monopartite. Chromatid breaks may result if one of the chromatids restituted after splitting (Evans 1962). Chromosome type aberrations resulted from the effect of radiation on chromosomes prior to splitting and chromatid types from the radiation effects on post split chromosomes. Nonorientation of the chromosomes found at metaphase is caused through the action of radiation on spindle fibres. However, multipolar mitosis resulted when spindle fibres are badly disrupted.

In the present observation paired (double) and single bridges were recorded at the anaphase stage of mitosis. Dicentric chromosomes are resulted from the union of two centric fragments forming a paired bridge at anaphase provided the two centromeres are pulled to the opposite poles. Yagyu and Morris (1957) reported a linear relation between radiation dose and chromosome aberration, particularly formation of anaphase bridges in tomato root tips. Double bridges may be seen at anaphase when there is translocation between non homologous chromosomes producing unlike arms. Single bridge occurred due to fusion of sister chromatid at a common breakage point. Another possibility may be suggested chromatid translocation when chromosomes became bipartite after gamma irradiation. High frequency of breakage or fragments are expected when more number of chromosomes are included in the breakage or fragmentation.

Meiotic chromosomal aberrations

Certain chromosomal anomalies were obtained in meiotic system by gamma rays treatment. The mode of action, quality and origin of these anomalies have been studied by a number of workers (Caldecott 1953, Caldecott and Smith 1952, and Bhaskaran and Swaminathan 1960). The type of induced aberrations depend
upon whether the chromosomes are effectively monopartite or bipartite at the time of radiation (Caldecott and Smith 1952).

Interchanges produced in plants grown from irradiated seeds indicate monopartite condition of the chromosomes at the time of irradiation. Reunion of broken ends occurs before the chromosomes became effectively bipartite (Lea 1955 and Swanson 1965).

In the present observations different numbers of univalents were exhibited at metaphase stage. Appearance of univalents indicate non pairing or early separation of chromosomes at the early or late stage of meiotic division respectively. At anaphase, bridge with fragment was recorded which is possibly due to the occurrence of paracentric inversion (Swanson 1965). Effect of radiation on spindle fibre may be the possible reason of the appearance of lagging chromosomes at anaphase.

Acknowledgements

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References