Gamma Ray Induced Chromosomal Interchange in Safflower

(Carthamus tinctorius L.)

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Summary  Safflower (Carthamus tinctorius L.) is a diploid crop having 2n=24 chromosomes. Two different plant types, heterozygous for reciprocal translocation (P-1 and P-8), showing marker phenotypic traits with weaker morphology and reduced seed formation as compared to their normal counterparts were isolated in an induced mutant population of a gamma irradiated set. The 2 induced translocation heterozygotes (P-1 and P-8) exhibited the formation of a ring or chain of 4 chromosomes along with 4 bivalents in the majority of PMCs at diakinesis and metaphase-I. The quadrivalents appeared both in ring and chain configurations but ring configuration was more frequent being 53.56% in P-1 and 48.82% in P-8. Besides this, other configurations like trivalents and univalents, along with variable numbers of bivalents, could also be observed at metaphase I. At anaphase-I, both the translocation heterozygotes showed various abnormalities as well: although some PMCs showed normal 12:12 separation, the most common and frequent separation to be encountered in this plant was 11:13 with 26.21% in P-1 and 28.17% in P-8. Besides this, bridges and laggards were also recorded as prominent abnormalities. Pollen fertility was found to decline to 50.55% in P-1 and 48.12% in P-8 against 98.00% in the control sets. The percentages of pollen abortion, seed formation and ovule fertility percentage were much higher in the first generation.

Key words  Translocation heterozygotes, Gamma rays, Safflower (Carthamus tinctorius L.), Pollen fertility.

Chromosomal breakage is a common phenomenon in plants and animals. When broken pieces of chromosomes are inserted elsewhere on the same chromosome or on a non-homologous chromosome, or when pieces may be exchanged, then it is called reciprocal translocation or chromosome interchange, or simply interchange (Mahama et al. 1999).

Occurrence of such structural modifications involving changes in chromosomal parts rather than in a set of chromosomes may be spontaneous but this is unusual. The frequency of such variations can be augmented artificially by treatment with mutagenic agents (Biswas and Biswas 2009). Among the various mutagenic agents known, radiation-induced chromosome breakage and the subsequent exchange of broken pieces between non-homologous chromosomes (reciprocal translocation) are the most frequent types of mutations in different crop plants (Burnham 1962, Sjödin 1971, Sadanaga and Newhouse 1982, Singh 2003). Among the various crop plants known, the genetics of reciprocal translocation has been studied by many workers viz. in soybean by Mahama et al. (1999), in Phaseolus vulgaris by Ashraf and Bassett (1987), in grass pea by Talukdar and Biswas (2006) and by Tripathi and Kumar (2009). The segregation pattern of these translocation heterozygotes has been lacking in safflower, however, due to poor staining ability and the problem of chromosome stickiness. Along with other structural chromosomal aberrations, reciprocal translocation served as an excellent cytogenetic tool for the identification and mapping of differ-

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ent linkage groups in plants (Sybenga 1996). It has also been involved successfully in transferring desirable traits (Sears 1956; Gustafsson 1965) and in generating different trisomics in a number of crop plants (Sutton 1939; Ramage 1960; Ashraf and Bassett 1987; Lakshmi and Nalini 1989; Auger and Birchler 2002). With these prospects in mind, the meiotic consequences of post-\(\gamma\)-irradiated \textit{C. tinctorius} plants were observed. Among the irradiated plants, 2 have been identified as structural heterozygotes.

Materials and methods

Experimental setup

Treatment with different doses of \(\gamma\)-rays (150 Gy, 300 Gy and 450 Gy) were made from the \(^{60}\text{Co}\) source at the National Botanical Research Institute, Lucknow, Uttar Pradesh, India to irradiate well-dried fresh seeds of \textit{Carthamus tinctorius} L. (Asteraceae). Seedlings were raised by sowing the irradiated and control (untreated) seeds in experimental pots.

Meiotic study

Cytological identification of translocation heterozygotes is based on meiotic chromosome association of translocated chromosome. For meiotic analysis, young floral buds were fixed in 3:1 solution of absolute alcohol and glacial acetic acid and later preserved in 70% alcohol. Slides were prepared via the standard acetocarmine squash technique using 2% acetocarmine.

Pollen study

Pollen fertility was scored by staining pollen grains collected from freshly opened flowers in 2% acetocarmine solution. The pollen grains showing lack of stainability were considered as sterile. Photomicrographs were taken from suitable preparations.

Results

As confirmed from the meiotic analysis, of the plants treated with gamma rays, 2 induced translocation heterozygotes were identified from \(M_1\) progeny as Progeny no. 1 (P-1) from 150 Gy and Progeny no. 8 (P-8) at 450 Gy doses of gamma ray treatment set.

The 2 induced translocation heterozygotes (P-1 and P-8), however, exhibited the formation of a ring or chain of 4 chromosomes along with 4 bivalents in the majority of PMCs at diakinesis and metaphase-I (Plate-1, Figs. 1–6). Besides this, other configurations like trivalents and univalents, along with variable numbers of bivalents, could also be observed. Table 1 gives an account of major metaphase-I configurations in both the translocation heterozygotes.

Out of the total 276 PMCs scored at metaphase-I in P-1, 50.71% PMCs displayed the presence of one quadrivalent with 4 bivalents while in the case of P-8 progeny out of 250 PMCs scored at metaphase-I, 55.13% PMCs displayed the presence of one quadrivalent with 4 bivalents as one of the most pre-dominant configurations (Table 1).

The quadrivalents appeared both in ring and chain configurations but ring configuration was more frequent being 53.56% in P-1 and 48.82% in P-8 (Table 2). The ring quadrivalents were of 2 types \textit{i.e.} adjacent and alternate. P-1 and P-8 had more adjacent orientation of quadrivalents than alternate ones.

Anaphase-I in both the translocation heterozygotes showed various abnormalities as well: although some PMCs showed normal 12:12 separation, the most common and frequent separation to be encountered in this plant was 11:13 with 26.21% in P-1 and 28.17% in P-8 (Table 2). Besides this bridges and laggards were also recorded as prominent abnormalities.

Pollen fertility was found to decline to 48.12% in P-8 against 98.00% in the control sets (Table 2).
Table 1. Different types of chromosomal associations (%) at diakinesis and metaphase-I in P-1 and P-8 translocation heterozygotes

<table>
<thead>
<tr>
<th>Types of associations</th>
<th>(%) in P-1 (150 Gy)</th>
<th>(%) in P-8 (450 Gy)</th>
</tr>
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<tbody>
<tr>
<td>12II</td>
<td>16.51</td>
<td>15.11</td>
</tr>
<tr>
<td>11II+2I</td>
<td>8.26</td>
<td>7.58</td>
</tr>
<tr>
<td>4H+1IV+2I</td>
<td>50.71</td>
<td>55.13</td>
</tr>
<tr>
<td>7II+2III+10I</td>
<td>30.16</td>
<td>27.77</td>
</tr>
<tr>
<td>8II+1III+5I</td>
<td>6.22</td>
<td>5.50</td>
</tr>
<tr>
<td>4II+2IV+4I</td>
<td>11.11</td>
<td>10.43</td>
</tr>
<tr>
<td>PMCs scored</td>
<td>276</td>
<td>250</td>
</tr>
</tbody>
</table>

Fig. 1. Complete translocation ring (8-shaped).
Fig. 2. Closed chain of multivalent at metaphase-II.
Fig. 3. Closed chain formation at anaphase-I.
Fig. 4. Multivalent formation at metaphase-I.
Fig. 5. Semi-circular chain of multivalents.
Fig. 6. Open chain of multivalents.
Discussion

Translocation heterozygotes have been of interest as they represent a model of surveying genic and chromosomal changes and provide novel gene linking by breaking undesirable associations (Tripathi and Kumar 2009). Chromosome interchanges involving the exchange of chromosome segments have been produced in a large number of plant species using ionizing radiation (Gill et al. 1980, Kumar and Singh 2003). These interchanges can be used for cytogenetic studies; if the chromosomes involved in an interchange are known, they can be arranged in a tester set (Gupta and Gupta 1991, Gupta 1995). Segmental interchanges have been reported in a number of plants like Capsicum (Meshram and Narkhede 1982), safflower (Singh et al. 1981), Lathyrus sativus (Biswas and Biswas 1999), Nigella (Saha and Datta 2000), and pearl millet (Kumar and Singh 2003). The association of chromosomes in rings and chains of 4, as well as the presence of bivalents and univalents, have suggested a double interchange involving 2 non-homologous chromosomes. Quadrivalents or higher chromosomal associations at metaphase-I indicate interchanges (translocations). The high frequency of ring quadrivalents at metaphase-I suggests that reciprocal translocation had occurred between 2 relatively large-sized chromosomes (Soriano 1957). On the other hand, the occurrence of chain quadrivalent has been attributed to complete terminalization at one end of the cross-shaped configuration, formed during pachytene (Kalloo and Das 1971).

Open chain formation is an outcome of the adjacent type of translocation followed by delayed segregation, and laggards may appear which ultimately give rise to micronuclei at telophase-II (Sinha and Acharia 1974).

The phenotypic similarity of the interchanged plants with the control plants indicated the possibility of no change in DNA in spite of chromosomal breakage (Sybenga and Wolters 1972). A high degree of pollen sterility observed in both semi-sterile translocation heterozygotes can be attributed not only to anaphase abnormalities but also to adjacent types of disjunctions. Pollen grains, due to unequal separation, bear higher or lower numbers thus leading to sterility. These pollens, however, if fertile may lead to aneuploid offspring and may give rise to some novel gene combinations.

Reciprocal translocations are important genetic aberrations to both the plant geneticist and the plant breeder. They are utilized for gene mapping (Cooper and Brink 1931), in transfer of desirable traits in wheat (Driscoll 1965), barley (Gustafsson 1965), and are good sources for creating aneuploids.

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References


Cooper and Brink 1931


