Gamma Ray and Ethyl Methane Sulphonate Induced Translocation and Inversion Heterozygote in *Lens culinaris* Medik (Lentil)

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**Summary** Seeds of *Lens culinaris* Medik (Lentil) were irradiated with various doses of gamma rays, (viz. 5 kR, 10 kR, 15 kR, 20 kR, 25 kR and 30 kR) and some seeds were also treated with different concentrations (0.1, 0.2, 0.3, and 0.4%) of Ethyl Methane Sulphonate (EMS) for 6 and 12 h in separate experiments. Two plants, one translocation heterozygote and one inversion heterozygote, were isolated from the population raised from 10 kR gamma ray-irradiated seeds and 0.2% EMS-treated seeds, respectively. The mutant plants displayed various types of chromosomal configurations at diakinesis/metaphase I, and anaphase/telophase I/II in meiosis. The translocation heterozygote exhibited the formation of ring/chain of four and six chromosomes in a majority of the PMCs at diakinesis/metaphase-I, and the inversion heterozygote was characterized by the presence of bridge and fragments at anaphase/telophase I/II because of various numbers and positions of crossovers in the inversion loop. Pollen fertility declined to 38% in the translocation heterozygote and 27.33% in the inversion heterozygote as compared to 96% in the control.

**Key words** *Lens culinaris* (Lentil), Gamma ray, EMS, Translocation heterozygote, Inversion heterozygote, Pollen fertility.

*Lens culinaris* Medik (Lentil) of family Fabaceae is one of the important and nutritious *rabi* pulses. It is an annually sown, self-pollinated, cool season food legume crop and diploid (*2n*=14) with a large genome size of approximately 4 Gb (Arumuganathan and Earle 1991). It derives the name *Lens* from the lens shaped seeds. Lentil can be grown as a secondary crop in dry land cereal-based rotations, because it is extremely good at nitrogen fixation. India ranks first in the world in respect of production (0.99 million tons) (FAO 2008). In India, lentil is mostly grown in the northern plains and central and eastern parts of India. The major lentil producing areas are situated in Madhya Pradesh, Uttar Pradesh, Bihar and West Bengal.

Induced mutagenesis has been recognized as the most efficient method for induction of morphological and genetic variabilities in plants, especially in those with limited genetic variabilities. Induction of mutations serves as a complimentary approach in genetic improvement of crop plants (Mehandjiev et al. 2001).

Chromosomal breakage by radiation is a common feature in both plants and animals. The broken pieces may be either inserted elsewhere in the same chromosome, or in a non-homologous chromosome, or pieces may be exchanged. When broken pieces of non-homologous chromosomes are exchanged, the product is termed as reciprocal translocation. Reciprocal translocations are important genetic aberrations to both the geneticist and plant breeders. Mahama et al. (1999) presented data that established the usefulness of reciprocal translocations in gene mapping.

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Reciprocal translocations have been used in transferring desirable traits in wheat (Driscoll 1965) and barley (Gustafsson 1965), and are good sources for generating aneuploids.

Chromosome structure rearrangement has played an important role in the evolution of plants (Stebbins 1971, King 1993), and meiotic pairing configurations have been used as primary evidence to reveal chromosome structure rearrangements (Singh 2003).

In the present study, *Lens culinaris* has been selected as a material due to its low chromosome number \(2n=14\) and relatively large size of chromosomes, and most importantly, it is used for commercial purposes. The present study mainly focuses on meiotic behavior of induced translocation and inversion heterozygotes of *L. culinaris*.

**Materials and methods**

Dry seeds of a local variety of lentil were irradiated with six doses (viz. 5 kR, 10 kR, 15 kR, 20 kR, 25 kR and 30 kR) of gamma rays at the Bhabha Atomic Research Center (B.A.R.C.) at Mumbai. Some seeds were also treated with EMS in the laboratory. For EMS treatment, dry seeds were presoaked for 2 h in tap water and dried with filter paper. Thereafter seeds were treated with four different concentrations (0.1, 0.2, 0.3 and 0.4%) of EMS for 6 and 12 h. After the treatment, seeds were washed under running tap water for 1 h and sown in pots along with control. For meiotic studies, young flower buds were collected from 40 randomly selected plants of irradiated and EMS-treated populations. Collected flower buds were fixed in freshly prepared Carnoy’s fixative 1:3 (acetic acid: absolute alcohol) for 24 h. Anthers of appropriate size were squashed in 2% iron-acetocarmine. Various stages of meiotic division were analysed and photographs were taken using the Leica microscope.

**Results**

*Meiotic division in control*

All the PMCs analysed at diakinesis/metaphase I had seven bivalents (Fig. 1). The number of chiasma/cell ranged from 11–16, mean being 13.85, of which 10.95 were terminalized, giving terminalization coefficient 0.75, and 2.9 chiasma was unterminalized. Chromosomes were equally distributed \(7:7\) towards each pole at anaphase/telophase-I and II (Fig. 7). Pollen fertility was 96%.

One translocation heterozygote was isolated from the population raised from 10 kR-irradiated seeds and an inversion heterozygote was isolated from 0.2% EMS-treated seeds.

*Translocation heterozygote*

Of the 100 PMCs analyzed in translocation heterozygote, 43% had a ring/chain of four chromosomes and five bivalents (Table 1, Figs. 2, 3). Out of 43 cells, 25 had a ring of four with five bivalents, and the remaining 18 showed a chain of four chromosomes with five bivalents (Table 1). Additionally, 26% had ring/chain of six chromosomes and four bivalents (Table 1, Figs. 4–6). Out of 26 cells, 9 had a ring of six and 17 cells had a chain of six chromosomes with four bivalents (Table 1). The average number of chiasmata per cell was 14.5, out of which 10.3 were terminalized, giving a terminalization coefficient of 0.71 in translocation heterozygote (Table 2). The pollen stainability in the heterozygous plant was 38% and seed set was very low.

*Inversion heterozygote*

The inversion heterozygote showed bridge/fragment configuration at anaphase/telophase-I/II in meiosis. At anaphase/telophase-I/II, 150 PMCs analysed in the plant were found to have various configurations of chromosomes confirming the presence of inversion heterozygote (Table 3). At anaphase/telophase-I, bridge, fragments and bridge+fragments were observed in 46.66% of PMCs (Figs. 8, 9). At anaphase/telophase-II, bridge, fragment, bridge with fragments and laggards were
observed in 38.66% of PMCs (Table 3, Figs. 10–12). Pollen fertility was studied in anthers taken from different buds. It was remarkably low in the abnormal plant (27.33%) as compared to the control (Table 3).
Mutation is known to enhance the genetic variability of crop plants. Since spontaneous mutations occur at very low frequency, induced mutations facilitate the development of new varieties at a swifter rate (Maluszynski 1990). In order to identify reciprocal translocation and inversion in heterozygous conditions, the chromosome configuration at diakinesis/metaphase-I and anaphase/telophase-I/II of the pollen mother cells were cytologically analysed.

Higher chromosomal associations (rings and chains of more than two chromosomes) at diakinesis/metaphase-I (Figs. 2–6) indicated the presence of translocation heterozygosity and chromosome bridges/fragments at anaphase-I/II (Figs. 8–12) indicated the presence of inversion heterozygosity.

Translocation heterozygote predominantly showed rings and chains of four and six chromosomes during diakinesis/metaphase I, which is due to single or double exchange between two or three nonhomologous chromosomes. Thus, the interstitial regions between the breakpoints and the centromere are small. Chiasma formation produces a ring of four chromosomes. The prevalence of ring interchange complexes may be because of the greater length of interchange parts as well as chiasmata associations in all the arms of interchanged chromosomes. If chiasmata are not formed in all the arms, a chain instead of a ring is formed (Shukla and Kumar 2009).

Verma and Goyal (2012) suggested that the high frequency of ring quadrivalents at metaphase-I is the confirmation of reciprocal translocation, which may occur between two relatively large-sized heterologous chromosomes. On the other hand, the occurrence of chain quadrivalents has been attributed to complete terminalization at one end of the cross-shaped configuration formed during pachytene (John 1990).

Induction of translocations through gamma rays is known in many plant species, e.g. Crotalaria juncea (Verma and Raina 1990), Vicia faba (Verma et al. 2004), etc. If the chromosomes involved in an interchange are known, these interchanges can be used for cytogenetic studies by arranging in a tester set (Gupta and Gupta 1991). Segmental interchanges have been reported in a number of plants like Nigella (Saha and Datta 2000), Glycine max (Kumar and Rai 2005) and Lathyrus sativus (Shukla and Kumar 2009). Translocation heterozygotes are of great interest as they provide a source for raising aneuploid offsprings with some novel gene combinations. They represent a model of surveying genetic and chromosomal changes and also provide novel gene linkage relations.

As for the inversion heterozygote in the present study, 46.66% of cells at anaphase/telophase I and 38.66% of cells at anaphase/telophase II showed bridge, fragment and bridge with fragment configurations. Bridge and fragment at anaphase/telophase I/II, arising from crossing over in

<table>
<thead>
<tr>
<th>Abnormalities</th>
<th>Number</th>
<th>Percent (%)</th>
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<tbody>
<tr>
<td>A. Anaphase/Telophase-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal cells</td>
<td>80</td>
<td>53.33</td>
</tr>
<tr>
<td>Bridges with fragments</td>
<td>57</td>
<td>38</td>
</tr>
<tr>
<td>Fragments</td>
<td>13</td>
<td>8.66</td>
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<tr>
<td>Total number of abnormal cells</td>
<td>70</td>
<td>46.66</td>
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<tr>
<td>B. Anaphase/Telophase-II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal cells</td>
<td>92</td>
<td>61.33</td>
</tr>
<tr>
<td>Bridges</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Fragments</td>
<td>11</td>
<td>7.33</td>
</tr>
<tr>
<td>Laggards</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Bridges with fragments</td>
<td>23</td>
<td>15.33</td>
</tr>
<tr>
<td>Total number of abnormal cells</td>
<td>58</td>
<td>38.66</td>
</tr>
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Inversion regions, may be an indicator of paracentric inversion heterozygote. Various possible types of configurations resulted from different numbers and positions of cross-over inside and outside the inversion loop. The presence of one bridge-fragment at anaphase-I was due to one cross-over inside the inversion loop. In the PMCs, where only the fragment was visible, it was presumed that this was due to the overlapping of the loop by other chromosomes at the poles and that the original configuration was loop-fragment (Bahl and Tyagi 1988).

In a paracentric inversion, two breaks occur in the same arm, so the inverted region does not include a centromere. In the present study, inversion heterozygote showed bridges, fragments and bridge+fragment configurations at anaphase-I and anaphase-II. A dicentric bridge and acentric fragment in low frequency at anaphase-I may arise due to an error in the normal process of crossing over. However, if the bridge and fragment are moderately constant, then this is most likely due to a paracentric inversion (Sjödin 1971).

In the inversion heterozygote plant, pollen sterility was found to be 72.66%. The most obvious genetic effect of inversion is the formation of imbalanced gametes that often causes microspore sterility (Shi-Quan et al. 2008).

References


