Cytogenetical Study of Induced Desynaptic Variants in *Phaseolus vulgaris* L.

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**Summary** The cytological screening of gamma ray treated plants revealed few desynaptic plants where an enhanced frequency of univalents were found at metaphase I along with few bivalents. The desynaptic plants were obtained at 200 Gy dose of gamma rays and were characterised as weak and medium strong type. The univalents remained unpaired till later meiotic stages and resulted in the unequal separation of chromosomes at anaphase I. The unequal separation of chromosomes as a consequence of desynapsis lead to the formation of micronuclei and gametes with imbalanced chromosomes, thus affecting the post-meiotic stages also. The desynaptic plants possess very few pods and acquire high pollen inviability. The study suggests the ability of gamma rays in the creation of male sterile lines in *Phaseolus vulgaris* L. The gamma rays either act on the genes responsible for synapsis and chiasma formation or disrupt the synaptonemal complex affecting chiasmatic dissociation. The desynaptic mutants could be used as a potential source for gathering information on chiasma maintenance mechanism as well as to study the genetic mechanism and consequences of male sterility in plants. Further, the male sterile lines could also be profitably utilized in hybridization breeding programmes to produce hybrid seeds.

**Key words** Desynapsis, Recombination, Chiasmata, Gamma ray, Male sterility, *Phaseolus vulgaris* L.

Meiosis is a specialized form of cell division that generates genetically diverse haploid gametes from diploid cells. It involves a single round of premeiotic S phase (meiS), followed by pairing and recombination between homologous chromosomes (Hua et al. 2013). Synapsis and recombination are the most significant meiotic events as they ensure proper chromosome segregation during meiosis I. The preliminary biochemical and cytological investigation revealed that several genes are required for homologous chromosome pairing and recombination (Roeder 1997, Zickler and Kleckner 1999). A mutation in the genes controlling the meiotic recombination may lead to failure or early dissolution or reduction of chiasma formation. At least one chiasma per bivalent is essential for orderly disjunction; otherwise some homologues may migrate to the same pole and form aneuploid gametes (Kumar and Rai 2006).

The mutations in the genes governing homologous chromosome pairing have been classified into two categories. Mutations that partially or completely prevent homologous chromosome pairing are classified as asynaptic mutants, while those that cause the premature separation of homologous chromosomes are classified as desynaptic mutants (Cai and Makaroff 2001). Such mutations, known respectively as asynapsis and desynapsis, will eventually lead to the formation of univalents. The frequency of univalents formed will depend on the degree to which such mutations influence the chromosome pairing (Falistocco et al. 1994). Spontaneous as well as induced desynapsis have been reported in different economical plants periodically, such as *Pisum sativum* (Gottschalk and Baquar 1971), *Hordeum vulgare* (Sharma and Reinbergs 1974), *Corchorus olitorius* (Basak and Paria 1980), *Capsicum annum* (Rao and Kumar 1983, Sengupta et al. 1999), *Cicer arietinum* (Kumar and Sharma 2001), *Haplopappus gracilis* (Jackson et al. 2002), *Glycine max* (Bione et al. 2002, Kumar and Rai 2006), *Corchorus fascicularis* (Maity and Datta 2009), and *Vicia faba* (Bhat and Wani 2015), etc.

Desynaptic plants open up new avenues for the production of aneuploid lines in breeding programmes. The desynaptic plants are used as a valuable resource for the study of male sterility in plants. Besides this, the study of the phenomenon of desynapsis also provides an information on the mechanism of chiasma formation and crossing-over. Considering these aspects, the present study has been undertaken to study the meiotic behaviour in the induced desynaptic plants in *Phaseolus vulgaris* L. through cytogenetical analysis and also to understand the genetic mechanism of male sterility.

**Materials and methods**

**Procurement of seeds**

Healthy and dry seeds of *Phaseolus vulgaris* L. variety PDR-14 (Uday) were obtained from Indian Institute of Pulses Research (IIPR), Kanpur, India.
Gamma ray irradiation

Seed packets containing 200 seeds per sample were prepared for the gamma ray irradiation. The seed samples were irradiated at different doses of gamma rays viz. 100, 200, 300, and 400 Gy, from a $^{60}$Co source at Nuclear Research Laboratory, IARI, New Delhi. The irradiated seeds were sown immediately in the field in replicates of three adopting a complete randomized block design (CRBD) to raise the M$_1$ generation. One set of untreated seeds were also sown in the field along with treated ones to act as control. At 200 Gy dose some plants were detected in which the frequency of pod formation was very low and were morphologically distinct from the control plants. The seeds obtained from these plants were sown in the next consecutive season to raise the M$_2$ generation.

Cytological studies

At the time of flowering, randomly selected young floral buds of appropriate size were fixed in freshly prepared Carnoy’s fluid (glacial acetic acid: alcohol in a 1:3 ratio) for 24 h. After fixation, the floral buds were...
transferred to 70% alcohol solution and stored at 4°C. The slides were prepared using the anther squash technique with 2% acetocarmine. Pollen fertility was determined by the acetocarmine–glycerine stainability test, where the darkly stained pollen grains with prominent nuclei and regular shape were considered as fertile and unstained and shrunken pollen grains with diminishing nuclei were considered as sterile.

### Results

In the M<sub>2</sub> generation, at 200 Gy dose of gamma rays five plants were found which were morphologically distinct from the control plants in having stunted growth and leathery type leaves. They displayed normal flowering but the pod formation in them was negligible. The cytological investigation in these plants revealed an abnormal meiotic pattern and reports of desynaptic behaviour in three plants. In the control plants, meiosis was found to be normal with 11 bivalents at diakinesis and metaphase I (Fig. 1A, B) and 11 : 11 segregation at anaphase I (Fig. 1C), while in the desynaptic plants a high frequency of univalents have been found (Fig. 1D–I). The bivalents were reported in a very low frequency in the induced desynaptic plants. Chromosome pairing was normal during pachytene stage in both control and desynaptic plants. The univalents ranging from 4-2, 8-6 and 14-10 were recorded both at diakinesis and metaphase I in the desynaptic plants. The descriptive configuration of chromosomes at diakinesis/metaphase I and segregation at anaphase I in the induced desynaptic plants of <i>Phaseolus vulgaris</i> L. have been presented in Table 1. The frequent configuration of bivalents and univalents in the induced desynaptic plants was (8-7) II+(8-6) I with the mean frequency of 22.60, 22.17, and 23.14% in plants numbered 1, 2, and 3, respectively, followed by (6-4) II+(14-10) I with the mean frequency of 19.31, 20.50, and 18.33%, respectively.

In the present study, the univalents tend to show a mutual dependence of position and are not found individually. After the dissociation of a bivalent, the univalents belonging to the same pair are preferably found in close proximity with each other. During metaphase I, the bivalents and univalents show alignment at the equatorial plate. However, in some PMCs the spindle apparatus was not clearly visible due to the random distribution of univalents.

The succeeding stages of meiosis are also profoundly affected as a consequence of univalent formation during desynapsis. During the commencement of anaphase I, the univalent chromosomes lagged in varying numbers (Fig. 1J). Besides this, the irregular separation of chromosomes, bridges, disturbed polarity etc. were also visible during anaphase I. Delay in chiasma terminalisation could be attributed as the prime factor responsible for the formation of laggards at anaphase I. The laggards which fail to reach the poles remain suspended in the cytoplasm and later developed into micronuclei during telophase I (Fig. 1K).

Meiotic irregularities were also found during the second meiotic division in the desynaptic plants. Chromosomal segregation at anaphase II was irregular with variable number of laggards, multipolarity etc. Variable number of laggards were visible during anaphase II. The percentage of meiotic aberrations in the induced desynaptic plants of <i>Phaseolus vulgaris</i> L. during each phase of meiosis have been given in Table 2. The mean abnormality percentage recorded was 85.80, 83.17 and 86.41% in plant number 1, 2 and 3, respectively. The irregular separation of chromosomes resulted in the formation of

### Table 2. Frequency of chromosomal aberrations during different stages of meiosis and pollen sterility in induced desynaptic plants of <i>Phaseolus vulgaris</i> L.

<table>
<thead>
<tr>
<th>Plant No.</th>
<th>Total number of PMCs observed</th>
<th>Meiotic stages (Abnormality %)</th>
<th>Mean abnormality (%)</th>
<th>Pollen sterility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diakinesis/Metaphase I configurations (%)</td>
<td>Segregations at anaphase I (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 (II)</td>
<td>(10-9) II+(4-2)I</td>
<td>(8-7) II+(8-6) I</td>
<td>(6-4) II+(14-10) I</td>
</tr>
<tr>
<td>1</td>
<td>483</td>
<td>4.55</td>
<td>11.52</td>
<td>22.60</td>
</tr>
<tr>
<td>2</td>
<td>477</td>
<td>3.63</td>
<td>13.20</td>
<td>22.17</td>
</tr>
<tr>
<td>3</td>
<td>485</td>
<td>6.19</td>
<td>14.35</td>
<td>23.14</td>
</tr>
</tbody>
</table>

Abbreviation: PMCs, Pollen mother cells; Dia, Diakinesis; Meta, Metaphase; Ana, Anaphase; Telo, Telophase.

### Table 1. Chromosomal configuration at diakinesis/metaphase I and segregations at anaphase I in induced desynaptic plants of <i>Phaseolus vulgaris</i> L.

<table>
<thead>
<tr>
<th>Plant No.</th>
<th>Total number of PMCs observed</th>
<th>Diakinesis/Metaphase I configurations (%)</th>
<th>Segregations at anaphase I (%)</th>
<th>Unequal separation (10 : 12, 8 : 14, 7 : 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>483</td>
<td>4.55</td>
<td>11.52</td>
<td>22.60</td>
</tr>
<tr>
<td>2</td>
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In plants numbered 1, 2, and 3, respectively, followed by (6-4) II+(14-10) I with the mean frequency of 19.31, 20.50, and 18.33%, respectively.
microspores of various sizes that alter the formation of normal viable pollen grains. Pollen fertility in the control plants was found as 96.33%. A high percentage of pollen sterility was found in the three induced desynaptic plants and was recorded as 81.64, 79.09 and 83.37% in plant 1, 2 and 3, respectively (Table 2).

Discussion

Desynapsis is the phenomenon in which the meiotic chromosomes pair at pachytene but begin to separate at diplotene and remain completely unpaired at diakinesis and metaphase I due to lack of chiasma formation (Rao 1975). Based on the univalent frequency amongst meioocytes, desynapsis can be distinguished as weak, medium-strong and complete type (Prakken 1943). On the basis of univalent and bivalent frequency at diakinesis and metaphase I, the three desynaptic plants obtained in the present study were classified as weak and medium-strong type.

Basically, the synaptic mutants are classified into two types on the basis of alignment of univalents and bivalents at metaphase stages. According to Peirson et al. (1997), in asynaptics, the univalents never align at the equator at metaphase I while in desynaptics, the bivalent as well as the univalents congregate at the metaphase plate. The congression of univalents at metaphase I in the present study suggested the desynaptic nature. Also, the univalents belonging to the same pair are found in close proximity with each other suggesting their recent disassociation.

Different reasons have been enumerated by several authors periodically to explain the occurrence of desynapsis. The influence of environmental factors on the expression of chromosomal pairing in desynaptic plants have been studied by Goodspeed and Avery (1939), Frakken (1943), Li et al. (1945), Soost (1951), Ahloowalia (1969), and Ray and Sherman (1988) etc. In the present study, the environmental factors were excluded as the probable causative factors because both the normal and desynaptic plants were grown under similar environmental conditions. It is very likely that gamma rays, the physical mutagen used in the present study, might have acted on the genes responsible for synapsis. Any alteration in synopsis and chiasma formation would lead to early dissociation of chiasmata resulting in the production of univalents. Generally, the replicated sister chromatids are adhered together by cohesion, a conserved multisubunit protein complex (Peters et al. 2008). Defects in synopsis not only result in recombination and chiasmata deficiency but it might also hinder establishment of sister chromatid cohesion necessary for chromosome segregation (Bickel and Orr-Weaver 1996). Moreover, recent studies also show that sporadic loss of chiasma maintenance in desynaptic plants also occurs as a result of defect in synaptonemal complex (Maguire et al. 1991, Caryl et al. 2000).

It is noteworthy that the phenomenon of chromosome pairing and recombination in plants are governed by genes. Mutation in any gene governing these phenomena would lead to disturbances in the chiasmata maintenance. According to Simchen and Stamberg (1969), a mutation in highly conserved rec gene system might lead to failure of chiasma formation and recombination. Similarly, Ji et al. (1999) proposed that recombination modifier mutation in rec gene might reduce recombination to a point where no pairing occurs. The major consequence of desynapsis is the formation of gametes with unbalanced chromosomes which eventually lead to the production of aneuploids. Besides aneuploids, different types of chromosomal aberrations such as laggards, micronuclei, precocious movement of chromosomes, unequal separation at anaphase etc. have also been reported in the induced desynaptic plants.

Desynapsis has been found to reduce male fertility in plants. The sterility encountered in the desynaptics could be associated with the homozygous condition of a single recessive gene (Gulfishan et al. 2013). The pleiotropic effects of the mutant gene(s) like breakage, stickiness and spindle abnormalities, will also contribute to pollen sterility (Thomas and Rajhathy 1966).

Conclusively, the cytological study clearly reveals that gamma rays induce male sterility in Phaseolus vulgaris L. The male sterile plants could be used as an important source in hybridization breeding programs to produce hybrid seeds. Moreover, the desynaptic plants will also give an insight on the chiasma maintenance mechanism to the breeders which could be profitably utilized in the breeding programmes for the improvement of crops.

Acknowledgements

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