Gamma Irradiation Induced Reciprocal Translocation in Pea (Pisum sativum L.)

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ABSTRACT: Seeds of Pisum sativum L. were treated with different doses (5, 10, 15, 20, 25, 30, 35 and 40 kR) of gamma rays. Three translocation heterozygotes were observed at meiotic division in a population raised from 20, 25 and 30kR treated seeds. The induced translocations showed a ring or chain of 4 chromosomes and 5 bivalents in most of the cells at diakinesis/metaphase I. The orientation of ring and chain quadrivalents predominantly showed the alternate type of orientation. The induced mutants showed delayed flowering, low flower number, low pod number as compared to control plants. The highest doses of gamma rays such as 35kR and 40kR caused death of all seedlings and no seed germination was observed.

KEYWORDS: Pisum sativum, Chromosome, Gamma irradiation, Translocation.

Pisum sativum L. (2n=14) a member of the family Fabaceae is one of the most important agriculture crops in the world as well as in India. The plant has been widely grown as a cool season vegetable crop, and consumed extensively worldwide as a rich source of protein, carbohydrates, vitamins and minerals important in human nutrition. Pisum sativum is an annual plant, with a life cycle of two to four months. It has a unique ability to improve the soil, like many legumes and contains symbiotic bacteria Rhizobia inside root nodules. As per new Angiosperm Phylogeny Group (APG) IV system (2016), the family Fabaceae contains about 766 genera and probably 19,580 species.

Interchanges are those structural changes in chromosome, where terminal segments of non-homologous chromosome have exchanged positions. These changes are also called reciprocal translocation. Interchanges in plants are usually associated with semi sterility of gametes; such semisterility is observed is only in those plants which have translocation in only one set of chromosomes, the other set being normal. These plants are called interchange heterozygotes. On the contrary, there can be plants which have same interchange in both sets of chromosomes. These would, therefore, be called interchange homozygotes.

The metaphase behavior of interchange (reciprocal translocation) multiples during meiosis has relevance to a wide range of disciplines, such as plant breeding, biological control of insect pests, cell biology and population cytogenetics (Rickards 1983). Many chromosome multiples have an extraordinarily high capacity for alternate orientation, and yet we have little clear understanding of why this is so (Lawrence 1963). Traditionally, explanation has been attempted by reference to certain morphological features of the multiple in question, such as symmetry in chromosome size and centromere position, chiasma frequency and location, and unspecified genotypic effects (Garber and Dhillon 1962).

The present study was aimed to induce mutations with the help of gamma radiations which produced three translocation heterozygotes whose behavior during meiosis has been presented here.

MATERIALS AND METHODS

The dry and healthy seeds of local cultivar of Pisum sativum have been used in the present study. The seeds were exposed to different doses of gamma rays (Viz. 5kR, 10kR, 15kR, 20kR, 25kR, 30kR, 35kR and 40kR) at the Bhabha Atomic Research Center, Mumbai. After irradiation, fifty seeds of each dose were sown in pots, ten in each. For meiotic study, young flower buds were fixed in 1:3 (acetic acid: absolute alcohol) mixture for 24h. The anthers of appropriate size were squashed in 2% iron-acetocarmine. Photographs were taken using temporary preparations with photographic microscope.

RESULTS

Control plants showed 7 bivalents (2n=14) at diakinesis/metaphase I in all the 50 PMCs observed (Table 2 and Fig. 1). Anaphase I distribution of chromosome was normal (7:7). Three translocation heterozygotes were isolated in the present study were categorized as PT₁, PT₂ and PT₃ respectively. One plant each of PT₁ and PT₂ translocation heterozygotes was isolated from the population raised from 20 and 25 kR, and PT₁ from 30kR irradiated seeds (Table 1). The PT₁ was characterized by the presence of a ring/chain of 4 chromosomes and 5 bivalents in 92% PMCs (Table 1, Figs. 3, 4). Rings of 4 were found in 39 and chains of 4 were found in11 cells (Table 2). The PT₂ was also characterized by the presence of a ring/chain of 4 chromosomes and 5 bivalents in 94% PMCs (Table 1, Fig. 5, 8). Rings of 4 were found in 35 cells and chains of 4 were found in 15 cells (Table 2). The PT₃ was characterized by the presence of a ring/chain of 4 chromosomes and 5 bivalents in 100% in PT₁ (Table 1, Figs. 6-11). Rings of 4 were found in 31 cells and chains
of 4 were found in 19 cells in PT₃ (Table 2). Anaphase I was observed in PT₃. The mean value of 5 bivalents is observed in most of the genotypes (Table 2). All three cytological variants showed delayed flowering, low flower number, low pod number as compared to control plants.

**DISCUSSION**

Interchanges may originate in nature and can be induced artificially through breakage and reunion among non-homologous chromosomes. In the present study, three induced reciprocal translocation heterozygotes of pea (*Pisum sativum*) were isolated from gamma-irradiated (20, 25 and 30 kR) seeds. The numerous reports on translocation heterozygotes in various plants such as *Pisum sativum* (Muller 1976; Verma and Goyal 2012), *Vicia faba* (Verma and Rao 1994), *Phlox drummondii* (Verma and Sharma 2000) and *Chrysanthemum carinatum* (Verma, et al. 2018).

In all three translocation heterozygotes (PT₁, PT₂ and PT₃), the number of ring was more than that of the chain quadrivalents. The configurations of the ring of four chromosomes at metaphase-I may be associated by means of the terminal or sub-terminal chiasmata. Two chains of four chromosomes at metaphase-I of meiosis, resulting from the failure of chiasma formation in one of the four arms of the cross shaped configuration of pachytene (Burnham 1962). Association of four chromosomes due to interchange heterozygosity (a quadrivalent) may have different orientations at metaphase I, which will result in adjacent or alternate segregations. Adjacent segregation results from a ring, where the homologous chromosome centromeres pass to opposite poles, while alternate segregations result from zigzag orientation at metaphase I. Alternate I and II segregation give rise to fertile gametes while adjacent I and II give rise to non-viable duplication and deficiency gametes. When these two types of segregations are of equal frequency, the result is semisterility (Endrizzi 1974).

In interchange heterozygotes at pachytene during meiosis, a cross-shaped configuration is observed and position of centre of cross will vary depending upon the position of breakpoints. However, rarely, the position of the centre of cross may vary in the same interchange due to pairing of non-homologous regions of chromosomes. Such variation can be explained, if pairing starts at the end of chromosomes or else if pairing starts both at the ends and centromeres of chromosomes but will not be possible if pairing starts from centromeres only and proceeds towards ends (Kasha and Burnham 1965). Burnham (1956) also suggested that pairing in non-homologous segments may be possible if a pairing is zipper like and begins earlier in one arm. According to him, the rates of pairing may also be variable. The three translocation heterozygotes obtained in the present study showed similarities in having interchange complex of 4 chromosomes of 2 non-homologous chromosome pairs (Figs. 3–12).

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**Table 1. Gamma rays treatment and percentage of the cells with interchange complex in translocation lines.**

| Translocation heterozygotes | Mutagen | No. of cells analysed | Cells with bivalent % | Interchange complex +5 bivalents % |
|-----------------------------|---------|-----------------------|-----------------------|-----------------------------------|
| PT₁                         | 20kR    | 50                    | 8                     | 92                                |
| PT₂                         | 25kR    | 50                    | 6                     | 94                                |
| PT₃                         | 30kR    | 50                    | 0                     | 100                               |

**Table 2. Frequency of ring and chain of four chromosomes in three induced translocation heterozygotes.**

| Genotype | No. of cells analysed | Ring of four No. Mean | Chain of four No. Mean | Ring + Chain of four No. Mean | No. of bivalent % Mean |
|----------|-----------------------|------------------------|------------------------|-------------------------------|------------------------|
| Control  | 50                    | ---                    | ---                    | ---                           | 7                      |
| PT₁      | 50                    | 39 0.78                | 11 0.22               | 50 1.00                       | 100 5                  |
| PT₂      | 50                    | 35 0.70                | 15 0.30               | 50 1.00                       | 100 5                  |
| PT₃      | 50                    | 31 0.62                | 19 0.38               | 50 1.00                       | 100 5                  |
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Figs. 1-12) Meiotic stages in control and translocation heterozygotes PT1-PT3. 1. Diakinesis in control; (7II), 2. Anaphase I (7:7) with bridge, 3-6. Ring of 4+5II in PT1 and PT2, 7. Ring of 4+4II+2Univ lent in PT3, 8-12. Chain of 4+5II in PT2 and PT3.
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