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Meiotic analysis of induced translocation heterozygotes in *Lepidium sativum* Linn.

G. Kumar*, P. Pandey, A. Pandey, J. Yadav, N.K. Tiwari and S. Pandey
Department of Botany, University of Allahabad, Prayagraj-211 002, India
***Corresponding Author Email :** kumar_girjesh@yahoo.com ***ORCiD:** https://orcid.org/0009-0006-7465-4439

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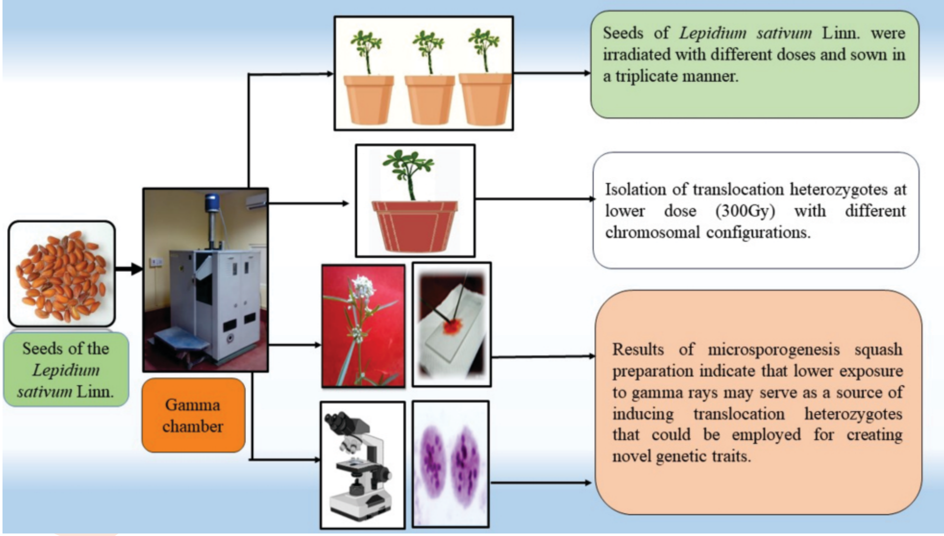
Abstract

Aim: To assess the cytological effects of Gamma rays on the pollen mother cells and post meiotic products of *Lepidium sativum* Linn. which resulted in isolation of translocation heterozygote and aneuploid gametes.

Methodology: During the experiment, parameters such as Meiotic stages, chromosomal structure, and pollen fertility were studied. Fresh seeds of Garden cress were orderly arranged in plastic and divided into four groups. The fresh seeds of each group were individually irradiated with 60Gy, 150Gy, 300Gy, 450Gy and 600Gy @ 7.247KGyh^{-1} in the Floriculture Laboratory of National Botanical Research Institute (NBRI), Lucknow (India). The source for gamma ray irradiation was Cobalt-60. The irradiated seeds were sown in triplicates along with their respective controls.



Seeds of *Lepidium sativum* Linn. were irradiated with different doses and sown in a triplicate manner.

Isolation of translocation heterozygotes at lower dose (300Gy) with different chromosomal configurations.

Results of microsporogenesis squash preparation indicate that lower exposure to gamma rays may serve as a source of inducing translocation heterozygotes that could be employed for creating novel genetic traits.

Results: Meiotic analysis of pollen mother cells (PMCs) at 300Gy showed translocation heterozygotes, however, in the control sets, the rate was negligible and phenotypically the plants were weak and short heighted. The cytological illustration of chromosomal configuration at diakinesis and metaphase I exhibited the superiority of tetravalents, bivalent and other forms of chromosomal associations such as multivalents. The induced translocation heterozygotes resulted in stickiness at metaphase and anaphase states along with the formation of bridges at anaphase stage. Due to chromosomal anomalies, the pollen fertility was reduced to 37.54±0.25% in contrast to control plants (98.20±0.16%).

Interpretation: The current research work would be of paramount interest as translocation heterozygote serves as a source for raising aneuploid offspring having novel genetic combinations.

Key words: Chromosomal anomalies, Heterozygotes, *Lepidium sativum* Linn, Meiosis, Pollen fertility

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Introduction

In plant breeding programs, genetic diversity is key for enhancing essential traits with high therapeutic value. Mutation breeding is one of the important tools for creating genetic variability by widening the gene pool and therefore, enhancement of qualitative traits for crop improvement and improving global food security by developing new crop varieties. Mutations are of various types that can be spontaneous and induced (Jorgensen *et al*., 2010). A "mistake" in DNA replication or mitosis causes a spontaneous mutation, whereas an induced mutation needs a mutagen, which might be physical, chemical, or biological (Kumar *et al*., 2022). Induced mutagenesis has escalated mutation breeding by developing new varieties with improved agronomic characteristics. Among physical mutagens, gamma radiation can be majorly used for creating genetic variation as it possesses no mass and, therefore, has high penetration power that can easily pass through biological materials (Karamker *et al*., 2021). Physical mutagens include ionizing radiations like x-rays and gamma rays, which act by generating a free radical to change molecules and cause single-strand breaks in DNA, leading to deletion and rearrangement (Gulfishan *et al*., 2012) as well as non-ionizing radiations like UV rays, which excite pyrimidine or purine molecules and cause the conversion of the AT to GC pair, leading to substitution mutation (Setlow *et al.* 1963). Chemical mutagens include EMS, MMS, 5-Bromouracil, and Colchicine, among which EMS was found to be the most potent mutagen that removes Guanine (G) from the chain and insertion of any base resulting in point mutation (Wani *et al*., 2017).

Plants embracing translocation known as heterozygotes are formed due to intersegmental changes between non-homologous chromosomes, among which one set is normal and the other set has unusual or different arrangements of chromosomes (Kumar *et al.,* 2003). Translocations are straightforward "cut and paste" structural genome variants that can develop when two coexisting breaks on separate chromosomes are repaired by joining the incorrect ends together (Ramsden and Nussenzweig, 2021). In plants and animals, chromosomal breakage is a common feature, if unable to restitute it might result in chromosomal inversion, *i.e.,* the formation of bridges at anaphase. The observed effects in the reciprocal translocation are the formation of multivalent associations at metaphase I and increased pollen, ovule sterility (Shaikh *et al.,* 2020), and the production of aneuploids with other chromosomal aberrations. Reciprocal translocation serve as a supremacy tool for identifying and mapping different linkage groups in plants (Sybenga *et al.,*1996; Pack *et al*., 1993).

Reciprocal translocation significantly contributes to the successful transfer of desirable traits (Gustafsson *et al*., 1965) and to produce different trisomics in several crop plants (Ashraf *et al.,* 1987; Lakshmi *et al.,* 1989). In the case of *Isotoma petraea*, translocation heterozygotes are the cause for preserving genetic heterozygosity (James *et al.,* 1965). In the absence of crossing-over in the interstitial segment, alternate orientation results in balanced chromosome combinations, and adjacent orientation results in unbalanced combinations (Sybenga *et al.,* 1967). *Lepidium sativum* L. (2n=16), commonly known as “garden cress” belongs to family Brassicaceae. It is an edible herb that grows upto 50 cm. *Lepidium sativum*. has pharmacological properties like antiasthmatic, anti-carcinogenic antibacterial, antioxidant and is also used for regulating menstrual cycle. The seeds contain edible oil mainly fatty acid alpha-linolenic acid (32-34.0%) (Diwakar *et al.,* 2010). The leaves of *Lepidium sativum* contain sulforaphane, glucosinolates,and flavanol compound that possess anti-inflammatory properties (Bell *et al.,* 2015).

The seeds of lepidium contain *i.e.,* Lepidine B & E alkaloid which possess antifungal property and can inhibit the growth of *Candida albicans* (Gacemi *et al.,* 2020). Paranjape *et al.* (2006) elucidated the use of garden cress as a traditional tonic to increase the height of children and milk content in females. On this current crop, a specialized cytological study was carried out by other researchers to examine the outcomes induced by various treatments. The effect of anti-microtubular agents (colchicine) during differentiation of statocytes in *Lepidium* roots and cytodifferentiation of polar plants has been reported by Hensel *et al.* (1986). Majeed *et al*. (2010) elucidated the effects of gamma rays on the cytological and morphological parameters of *Lepidium*. Similarly, Aqafarini *et al.* (2019) reported induction of tetraploidy in garden cress due to colchicine treatment.

Due to narrow genetic base of *Lepidium sativum*, conventional breeding stages such as mass selection, pure line selection, and recurrent selection does not serve as a potential tool to obtain desirable traits/genetic variations which are required for crop improvement. Convention breeding requires a lot of time and effort and involves breeding among two plants that can sexually mate with each other, and this limits the production of new traits that can enhance the quality of crops. Also, seeds become expensive which is difficult for impoverished farmers to afford as it requires a lot of time to maintain the pure lines from F1 hybrids (Ahmar *et al*., 2020). As *Lepidium sativum* consist of volatile oils with pungent odor, et is used to treat respiratory disorders, muscular pain, inflammation, and bone fractures. In order to obtain higher yield contributing varieties and to fulfill the demand of global market, mutation breeding is required in *Lepidium sativum*. Translocation heterozygotes having the potential for preserving genetic heterozygosity, as in the case of *Isotoma petraea* (James *et al.,* 1965) are generally identified by reduced reproductive ability and the presence of multivalents during meiosis (Sharma *et al.,* 2011). In view of the above, in the present study the cytological effects of Gamma rays on the pollen mother cells and post-meiotic products of *Lepidium sativum* Linn. were examined, which resulted in the isolation of translocation heterozygote and aneuploid gametes.

Materials and Methods

Healthy and fresh seeds of *Lepidium sativum* L. were obtained from Directorate of Medicinal and Aromatic Plant Research (DMAPR) of Gujarat Center, India.

The designed experiment was performed in the Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Prayagraj, India, during Rabi season, October (2022). The fresh seeds of *Lepidium sativum* were systematically arranged in plastic or poly bags and divided into four groups along with one control set. The fresh seeds of each group were individually irradiated with Cobalt-60, that as a served source of gamma rays in the Floriculture Laboratory of the National Botanical Research Institute (NBRI), The selected doses for irradiation were 150, 300, 450, and 600Gy @ 7.247KGy h^{-1}. Thereafter, the seeds were sown in triplicate along with their respective controls in respective pots in the field of Botany Department, University of Allahabad, following complete block random design (CBRD).

For performing meiotic studies, young floral buds were fixed in freshly prepared Carnoy's fixative for 24 hr and stored in 90% ethanol in refrigerator for further studies. Meiotic slides were prepared using squash technique with 2% acetocarmine (Dubrovsky and Contreras-Burciaga, 1998). Meiotic slides were analyzed and suitable cells were photographed with a Nikon research photomicroscope. Chromosomes were at Metaphase I as rings, chains, and multiple chromosomal associations. Pollen fertility was also calculated using a 2 % acetocarmine stain. Pollen grains with undersized and stained nuclei were considered fertile while unstained pollen grains lacking nuclei were recorded as sterile.

Statistical analyses: Statistical Analyses was performed by using SPSS 16.0 software. A One- way analysis of variance (ANOVA) and Duncan's Multiple Range test DMRT ($p<0.05$) were performed for mean separation. By using Sigma Plot 10.0 software the graphs were plotted. The actual mean and standard error were calculated and the data were subjected to analysis of variance.

Results and Discussion

Exposure of plant materials to physical mutagens, especially on seeds, causes mutagenic changes in live cells via various mechanisms. After exposure, there are two possible mechanisms: the primary or physical response, which manifests as molecular disruption, and the secondary or chemical response, which results from the ionized molecules and produces free radicals (Lagoda *et al*., 2012). As gamma radiation has the lowest wavelength and highest energy, therefore its penetration power is high that causes mutagenic changes leading to severe alterations in the living cells. These alterations result in the formation of reactive oxygen species (hydrogen peroxide, hydroxyl ions) that cause high functional changes in the metabolic activities (Majeed *et al.,* 2017; Majeed *et al.,* 2018). Gamma radiation have high electron volts of energy that serves as the utmost mutagen known for inducing high mutation and variability (Kovacs *et al.,* 2002). After exposure to gamma radiation, during cytological analysis, translocation heterozygote was observed in one of the plants at 300 Gy dose and it was reported for the first time in *Lepidium sativum* Linn. Translocation heterozygote will serve for raising aneuploid progeny/ male sterile line having some novel genetic traits. Since, translocation occurs due to pairing between non-homologous sets, which is an unusual feature, therefore it results in ring formation at metaphase I, which is cytologically detectable. After meiosis, three types of gametes are formed, *viz.,* normal, balanced and unbalanced. Therefore, semi-sterile individuals is a unique feature for creating variations in the *Lepidium* which may be utilized by plant breeders to develop mutant lines. Gamma irradiations have been successful in inducing translocation heterozygosity in plant species like *Pennisetum typhoids* (Pantulu *et al.,* 1967), *Vicia faba* (Sjodin *et al.,* 1971), *Pisum sativum* (Verma *et al.,* 2012), Safflower (Verma *et al.,* 2014), *Vinca rosea* (Sudhakaran *et al.,* 1971), *Plantago* (Padha *et al.,* 1998) and papaver somniferum (Kumar *et al.,* 2012).

Cytological studies which include normal and different anomalous cellular stages and the rate of pollen fertility were observed in microspore mother cells and pollens of *Lepidium sativum* Linn. respectively. Meiosis was normal and regular, exhibiting 8 bivalents at diakinesis (Fig. 1 A) and metaphase I.Chromosomes showed normal separations (8:8) at anaphase in the case of control plants, followed by normal meiotic stages and perfect formation of tetrads. Erstwhile in gamma-irradiated plant populations one plant in 300 Gy set was procured and observed as deciphering translocation heterozygotes remarked based on the morphological and cytological parameters viz. with lower seed yield, delicate stem and breakage and reunion of non-homologous chromosomes as compared to features of control plants. Moreover, translocation heterozygotes were characterized by the presence of ring or chain configurations in the pollen mother cells at diakinesis-metaphase I that could be differentiated from the normal ones. At a lower dose (150 Gy) translocation was not reported, and chromosomal anomalies were reported. At the highest dose *i.e*. 600 Gy germination was not reported while at 450 Gy the plants did not survive up to the budding stages. At 300 Gy dose and various chromosomal variations, translocating heterozygotes were isolated in one plant. Hence, data pertaining to translocation heterozygotes at 300 Gy was given in the present manuscript. Chromosomal anomalies such as laggards, bridges, and various multiple associations including univalent were observed during the cytological study of translocation heterozygote that showed various rings and chain configurations (Fig. 1D-I). Chromosomal separation during anaphase is prevented by uneven translocation, chromosome segment inversions, and the formation of chromatin bridges due to stickiness (Evseeva *et al*., 2005; Bouzekri *et al.*, 2023).

Major variations in chromosomal configurations at metaphase I is demonstrated in Table 1. The meiotic studies showed different chromosomal configurations such as PMCs structurally with ring configurations were considered as bi ring showing 8-shaped and open ring. PMCs occupying zigzag patterns or side-by-side orientations were identified as chains (Fig. 1D-I). The majority of PMCs showed the presence of one or more quadrivalents and bivalents, alongwith trivalent, pentavalent, and decadent configurations were also associated. Translocation heterozygotes include association of

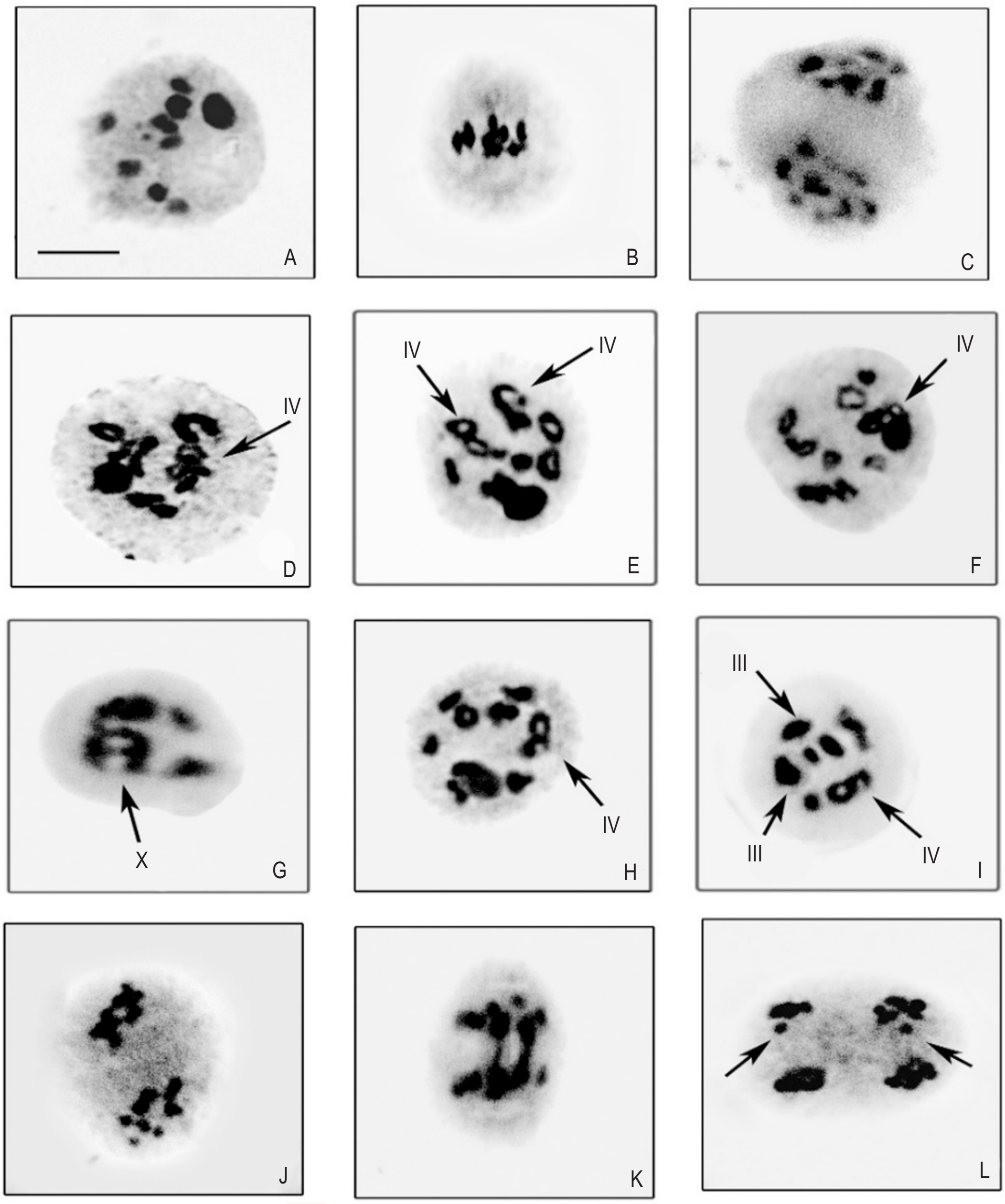

Fig. 1: Meiotic stages in *Lepidium sativum*: (A) Diakinesis (8 bivalent with nucleolus); (B) Normal Metaphase (8 bivalents); (C) Normal Anaphase (8:8 separation); (D-I) Chromosomal configurations at Metaphase I of Translocation heterozygote (arrows showmultivalent); (D) 1IV + 5II + 2I; (E) 2IV + 2II+ 4I (F) 1IV + 4II + 4I; (G) 1X + 3II; (H) 1IV + 5II + 2I; (I) 1IV + 2III + 2II + 2I; (J) Unequal segregation at anaphase; (K) Double bridge formation at anaphase; (L) 2 laggard formation at anaphase II; Scale bar= 5µm.

chromosomes in rings and chains of octavalents as well as bivalents and univalents, indicating a double interchange complex involving four non-homologous chromosomes and the position of centromere oriented alternately or adjacently (Kumar *et al.,* 2019). The typical ring interchange complex may result from chiasmata linkages in all chromosomal arms and the large length of the interchanged portions.

Chains are formed when both the pieces of chromosomes are short or due to presence of break sites near the centromere and if there is no development of chiasmata in both arms (Datta *et al.,* 2003). During the cytological study of induced translocation heterozygote, a total of 192 PMCs were manifested at diakinesis and metaphase I, out of which 5II + 1IV + 2I was highly ubiquitous in maximum PMCs (19.79% followed by 2 II + 1

Table 1: Various chromosomal configurations in 192 PMC's and their frequencies at metaphase I of translocation heterozygote

Meiotic configuration	Number of pollen mother cells	Frequency %
5II + 6I		3216.66
5II + 1IV + 2I	38	19.79
4 II + 4 I	14	7.29
2II + 2IV + 4 I	21	10.93
4II + 1IV + 4 I	16	8.33
3II + 1 X	7	3.64
3 IV + 2 II	12	6.25
2 II + 1 VIII + 4 I	26	13.74
2 II+ 1 X + 2 I	18	9.37
2II + 1 IV + 2 III + 2I	9	4.68

Table 2: Chromosomal associations in 205 PMCs and pollen fertility of translocation heterozygote

Dose	Rings (%)		Chains (%)		Adjacent orientation (%)	Alternate orientation (%)	Pollen fertility (Mean ±S.E.)
	Open	Bi-rings 8-shaped	Adjacent	Zig-zag			
Control	-	-	-	-	-	-	98.20±0.16
300 Gy	38.23	25.82	8.33	28.33	46.56	54.15	37.54±0.25
Total	64.01	36.66					

Table 3: Frequency of types of segregations at the translocation heterozygote

Types of segregation	Number of pollen mother cells	Frequency (%)
8: 8	48	31.16
Unequal segregation	52	33.76
Laggards	35	22.72
Bridges	19	12.33
Total	154	
Types of Segregation	No. of PMCs	Frequency (%)
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Unequal Segregation	52	33.76
Laggards	35	22.72
Bridges	19	12.33
Total	154	

VIII + 4 I, (13.74%) and 2II + 2IV + 4 I (10.93%). In some PMCs, the univalent formation was also recorded in a higher frequency, which might be the outcome of an exchange of interstitial chromosomal translocation that does not include the chromosomal end. Due to such interstitial differences, the chromosomes do not form rings or chains but pair loosely at pachytene and desynapse at Metaphase I (Kumar and Singh, 2003; Kumar and Singh, 2020). The lowest frequency of 3.64% was shown by 3II + 1 X configuration (Table 1). The meiotic configuration showed (Table 2), the dominance of rings (64.01%) over the chains (36.66%), among which open rings and bi-rings were approximately 38.23% and 25.82%, respectively. Hence, a clear prevalence of alternate orientation (54.15%) was observed over adjacent orientation (46.56%). Adjacent orientation was related to open and adjacently attached chromosomal arms, while alternate orientation was a consequence of zig-zag pattern and bi-rings (8 shaped). Orientation behaviors, *i.e.,* alternate and adjacent interchange complexes were also studied by analyzing the meiotic configuration of translocation multiples at metaphase I (Table 2). Translocation heterozygosity is a boon for retaining heterozygosity but is also responsible for reduced fertility due to chromosomal non-disjunction (Bloom *et al.,* 1974). The alternate orientation of centromere and their subsequent disjunction at anaphase is positively correlated with fertility resulting in balanced gametes (Van *et al.,* 1974) and formation of 8 shaped chromosomes. Alternate orientation showing quadrivalents result in unequal separation leading to the production of unbalanced or non-viable gametes due to duplication and deficiency of genes (Ghosh and Datta, 2006). Similar results were also observed in nine different plant species like *Artemisia annua*, *Datura stramonium*, *Diclipterabu pleuroides*, *Hemigraphis latebrosa*, *Leycesteria formosa, Salvia plebeia, Senecio nudicaulis, Sonchus brachyotus*, and *Verbena officinalis* from the Kangra district showing high pollen fertility due to the formation of multivalents with alternate-disjunction from Kangra district, Himachal Pradesh (Bala *et al*., 2016). Equal frequency of adjacent and alternate configurations of interchange complex at metaphase I generate semi-sterility in plants like maize, sorghum and pea (Ghaffari *et al*., 2009).

According to Hagberg (1954), chromosomes with non-co-orientation in the translocation heterozygotes result in duplicate-deficient gametes following 2:2 segregation or aneuploid following 3:1 segregation. In maximum number of PMCs, the Anaphase I was found to be regular. However, other anomalies were also observed like unequal separation (33.76%), laggards (22.72%) (Fig.1J, 1L), and bridge formation (12.33%). Abnormalities like laggards, bridges, and micronuclei are the consequence of unequal or difficulty in accurate segregation of chromosomes at Anaphase I/II of Telophase I/II. High possibility of formation of laggards presenting unequal and delayed separation is mainly contributed by alternate orientation (Kumar *et al.,* 2012). Elevated meiotic disturbances via unequal segregation of the number of translocated chromosomes and the consequent increased pollen fertility are the consequences of the involvement of a greater number of different non-homologous chromosomes. This study shows induced translocation heterozygote of *Lepidium sativum* presenting the exchange of chromosomal segments playing a crucial role that serves as a model of genetic and cytological changes. The meiotic configuration of interchange multiples is affected by factors such as the morphology of translocated part, chiasma position and number, degree of chiasma terminalization, and arm ratio (Sybenga, 1968; Verma and Raina,1990; Shaikh *et al.,* 2020). The meiotic disjunction of interchange chromosomes is important as it regulates the fate of the cell.

Translocation heterozygotes serve as an important tool in the plant breeding programs and also, these interchange heterozygotes are considered as a source for raising aneuploid progeny having new genetic traits. This study reports translocation heterozygosity in *Lepidium sativum* along with other meiotic abnormalities to exhibit the extent of low fertility post exposure gamma rays and further to assess if sterility would be passed on to the successive generation or remain as recessive. Therefore, it is necessary to identify all the translocated chromosomes for their utilization in achieving novel gene combinations and for classical and molecular linkage studies in *Lepidium sativum*.

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Authors' contribution: G. Kumar: Checked and finalized the manuscript; **P. Pandey:** Prepare the whole write-up of the manuscript; **A. Pandey:** Prepare the statistical data and tables; **J. Yadav:** Prepared and configurated cytological plates; **N.K. Tiwari, S. Pandey:** Formatting of manuscript.

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References

Ahmar, S., R.A. Gill, K.H. Jung, A. Faheem, M.U. Qasim, M. Mubeen and W. Zhou: Conventional and molecular techniques from simple breeding to speed breeding in crop plants: recent advances and future outlook. *Int. J. Mol. Sci.*, **21**, 2590 (2020).

Anindita, G. and A.K. Datta: Gamma-rays induced reciprocal translocation in *Nigella damascene* L. (Love-in-a-mist). *Caryologia*, **59**, 31-36 (2006).

Aqafarini, A., M. Lotfi, M. Norouzi and G. Karimzadeh: Induction of tetraploidy in garden cress: morphological and cytological changes. *Plant Cell, Tissue Organ Cult.*, **137**, 627-635 (2019).

Ashraf, M. and M.J. Bassett: Five primary trisomics from translocation heterozygote progenies in common bean, *Phaseolus vulgaris* L. *Theor. Appl. Genet.*, **74**, 346-360 (1987).

Auerbach, C.: Chemically induced mosaicism in *Drosophila melanogaster*. *Proc. R. Soc. Edinb, Section B: Biol. Sci.*, **62**, 211-222 (1946).

Bala, S., R. A. Malik and R. C. Gupta.: Male meiosis in structural heterozygotes. *Biolife*, **4**, pp. 587-594 (2016).

Bell, L., M.J. Oruna-Concha and C. Wagstaff: Identification and quantification of glucosinolate and flavonol compounds in rocket salad (*Eruca sativa, Eruca vesicaria* and *Diplotaxis tenuifolia*) by LC–MS: Highlighting the potential for improving nutritional value of rocket crops. *Food Chem.*, **172**, 852-861 (2015).

Bloom, W.L.: Origin of reciprocal translocations and their effect in *Clarkia speciosa*. *Chromosoma*, **49**, 61-76 (1974).

Borgohain, A., P. Sonowal, N.Borah and P. Chamuah: Mutation and its significance in sericulture industry: a review. *J. Exp. Zool. Ind.*, **25**,1597-1600 (2022).

Chatterjee, N. and G.C. Walker: Mechanisms of DNA damage, repair, and mutagenesis. *Environ. Molec. Mutage.*, **58**, 235-263 (2017).

Chaudhary, J., R. Deshmukh and H. Sonah: Mutagenesis approaches and their role in crop improvement. *Plants (Basel),* **8**, 467 pages (2019).

Datta, A.K. and A. Saha: Cytomorphological studies and seed protein characterization of *Nigella sativa* L. and *Nigella damascena* L. *Cytologia*, **68**, 51-60 (2003).

Diwakar, B.T., P.K. Dutta, B.R. Lokesh and K.A. Naidu: Physicochemical properties garden cress (*Lepidium sativum*) seed oil. *J. Am. Oil Chem. Soc.,* **87**, 539-48 (2010).

Dubrovsky, J. G. and L. Contreras-Burciaga: A squash preparation method for root meristem field studies. *Biotech. Histochem.,* **73**, 92-96 (1998).

Gacemi, S., K. Benarous, S. Imperial and M. Yousfi: Lepidine B & E as new target inhibitors from *Lepidium sativum* seeds against four enzymes of the pathogen *Candida albicans*: *in vitro* and *in silico* studies. *Endocr. Metab. Immune. Disord. Drug. Targ.,* **20**, 127-138 (2020).

Ghaffari, S.M., G. Karimzadeh and A. Najafi: Occurrence of reciprocal translocation in *Lathyrus boissieri* Sirj (Fabaceae) from Iran. *Cytologia*, **74**, 195-199 (2009).

Ghosh, A. and A.K. Datta: Gamma-rays induced reciprocal translocation in *Nigella damascene* L. (Love-in-a-mist). *Caryologia*, **59**, 31-36 (2006).

Gulfishan, M., A. H. Khan, I. F. Jafri and T. A. Bhat: Assessment of mutagenicity induced by MMS and DES in *Capsicum annuum* L. *Saudi J. Biol. Sci.*, **19**, 251-255 (2012).

Gustafsson, A.: Charcteristics and rates of high productive mutants in diploid barley. In The use of induced mutations in plant breeding. FAO/IAEA Technical Meeting, Rome, pp. 323-337(1965).

Hensel, W.: Cytodifferentiation of polar plant cells Use of anti-microtubular agents during the differentiation of statocytes from cress roots (*Lepidium sativum* L.). *Planta,* **169**, 293-303 (1986).

James, S.H.: Complex hybridity in Isotomapetræa. *Heredity,* **20**, 341-353 (1965).

Jorgensen, E.D., H. Zhao, F. Traganos, A.P. Albino and Z. Darzynkiewicz: DNA damage response induced by exposure of human lung adenocarcinoma cells to smoke from tobacco-and nicotine-free cigarettes. *Cell Cycle,* **9**, 2170-2176 (2010)

Karmaker, N., K. M. Maraz, F. Islam, M.M. Haque, M. Razzak, M. Z. I. Mollah and R.A. Khan: Fundamental characteristics and application of radiation. *GSC Advanced Research and Reviews*, **7**, 064-072 (2021).

Kovacs, E. and A. Keresztes: Effect of gamma and UV-B/C radiation on plant cells. *Micron*, **33**, 199-210 (2002).

Kumar, G. and S. Naseem: Genetic behaviour of induced translocation heterozygote in poppy. *Cytologia*, **77**, 425-430 (2012)..

Kumar, G. and A. Pandey: Cytogenetical investigation of a translocation heterozygote induced by gamma rays in *Coriandrum sativum* L. *Cytologia,* **84**, 211-214 (2019).

Kumar, G. and R. Singh: Genetic behavior of induced translocation heterozygote in *Artemisia annua* L. Electron. *J. Plant Breed.,* **11**, 854-859 (2020).

Kumar, G. and V. Singh: Meiotic behaviour of induced translocation heterozygote in pearl millet (*Pennisetum typhoides*). *Caryologia*, **68**, 245-248 (2003).

Lagoda, P.J.L.: Effects of radiation on living cells and plants. In: Plant Mutation Breeding and Biotechnology. Wallingford UK: CABI, pp. 123-134 (2012).

Lakshmi, N. and P. Nalini: Tertiary trisomic in *Capsicum annuum* L. *Cytologia,* **54**, 395-399(1989).

Leehan, J. D. and W. L. Nicholson: The spectrum of spontaneous rifampin resistance mutations in the *Bacillus subtilis* rpoB gene depends on the growth environment. *Appl.Environ. Microbiol.*, **87**, e01237-21 (2021).

Majeed, A. and Z. Muhammad: Gamma irradiation effects on some growth parameters of *Lepidium sativum* L. *World J. Fungal Plant Biol.,* **1**, 8-11 (2010).

Majeed, A., Z. Muhammad, R. Ullah, Z. Ullah, R. Ullah, Z. Chaudhry and S. Siyar: Effect of gamma irradiation on growth and post-harvest storage of vegetables. *PSM Biol. Res.*, **2**, 30-35 (2017).

Majeed, A., Z. Muhammad, R. Ullah and H. Ali: Gamma irradiation i: effect on germination and general growth characteristics of plants–a review. *Pak. J. Bot.,* **50**, 2449-2453 (2018).

Pack, S.D., P.M. Borodin, O.L. Serov and J.B. Searle: The X-autosome translocation in the common shrew (*Sorex araneus* L.): late replication in female somatic cells and pairing in male meiosis. *Chromosoma*, **102**, 355-360 (1993).

Padha, L., S. Sareen, R. Mangotra and A.K. Koul: Genetic diversity among Plantagos. XXXIII: Interchanges in Plantago ovate Forsk. *Caryologia*, **51**, 149-158 (1998).

Pantulu, J.V.: Chromosomal alterations in pearl millet induced by γ-rays. *Nature*, **213**, 101-102 (1967).

Paranjape, A.N. and A.A. Mehta: A study on clinical efficacy of Lepidium sativum seeds in treatment of bronchial asthma. *Iranian J. Pharmacol. Ther.,* **5**, 55-59 (2006).

Ramsden, D. A. and A. Nussenzweig: Mechanisms driving chromosomal translocations: Lost in time and space. *Oncogene*, **40**, 4263-4270 (2021).

Setlow, R. B., P. A. Swenson and W. L. Carrier: Thymine dimers and inhibition of DNA synthesis by ultraviolet irradiation of cells. *Science*, **142**, 1464-1466 (1963).

Shaikh, N., R.C. Verma, P. Dass and M.A. Khah: Meiotic investigation in reciprocal translocation heterozygotes of *Commelina benghalensis* L. (Commelinaceae) induced by gamma irradiations. *Caryologia,* **85**, 213-217 (2020).

Sharma, G. and R.N. Gohil: Occurrence of differential meiotic associations and additional chromosomes in the embryo-sac mother cells of *Allium roylei* Stearn. *J. Genet.,* **90**, 45-49 (2011).

Sjödin, J.A.N.: Induced morphological variation in *Vicia faba* L. *Hereditas*, **67**, 155-179 (1971).

Sudhakaran, I.V.: Meiotic abnormalities induced by gamma rays in *Vinca rosea* Linn. *Cytologia,* **36**, 67-79 (1971).

Sybenga, J.: Aneuploid and other cytological tester sets in rye. *Euphytica*, **89**, 143-151 (1996).

Sybenga, J.: Interchromosome effects on chiasma frequencies in rye. *Genetica*, **38**, 171-183 (1967).

Sybenga, J.: Orientation of interchange multiples in Secale cereale. *Heredity*, **23**, 73-79 (1968).

Van, H.C.: Meiotic disjunction, sex-determination and embryonic lethality in an X-linked “simple” translocation of the onion fly, *Hylemya antiqua* (Meigen). *Chromosoma*, **47**, 45-60 (1974).

Verma, R.C. and S. Goyal: Induced reciprocal translocation in *Pisum sativum* L. *Cytologia*, **77**, 485-489 (2012).

Verma, R.C. and P. Shrivastava: Radiation-induced reciprocal translocations in safflower (*Carthamus tinctorius* L.). *Cytologia,* **79**, 541-545 (2014).

Verma, R. C. and S. N. Raina: Cytogenetics of Crotalaria. IV. Induced translocation lines in *C. juncea. Nucleus (Calcutta)*, **33**, 11-14 (1990).