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Research Article

Genetic behavior of induced translocation heterozygote in *Artemisia annua L.*

Girjesh Kumar and Rajani Singh*

Plant Genetics Laboratory, Department of Botany, University of Allahabad, Allahabad-211002, India
E-Mail: singh.rajani1995@gmail.com

Abstract

From the population raised from gamma irradiated seeds at different doses (100, 200 and 300 Gy), at 100 Gy phenotypically dissimilar plant (from the control plants) was isolated. The plant was shorter and morphologically weaker in contrast to control population. The cytological studies were carried out in a plant showed segmental exchanges between heterologous chromosomes. Chromosomal associations in rings and chains along with bivalents and univalents were observed in pollen mother cells (PMCs) during microsporogenesis. Various anaphasic anomalies were also taken into account including chromatid bridges, laggards and unequal separations *etc.* Subsequent stages of meiosis were also found to be highly intermittent. Owing to the high frequency of chromosomal aberrations, pollen fertility in control (96.32%) were efficaciously reduced to 38.26% at dose of 100Gy. The present study has been conducted to assess cytological behavior of the induced translocation heterozygote which is first time induced through gamma irradiation in the *Artemisia annua* L.

Key words

Artemisia annua L., Chromosomal aberration, Heterozygote, Pollen fertility, Translocation

INTRODUCTION

Chromosomal breakage is a common phenomenon reported in both animals and plants. During male meiosis translocation heterozygote's were formed by the interchange of segment between sister chromatids of non-homologous chromosomes which leads to the formation of combination of original and novel chromosomes set in one system. Chromosomal exchanges are of prime importance because of the opportunities they offer for the study of chromosomal behaviors. Therefore, an individual carrying a translocation segment of one linkage group and the breakpoint is the attachment of a broken segment (Talukdar 2013). The observed effects of such abnormalities include the non-transmission of gametes through pollen and increased pollen and ovule sterility of plants (Phillips1978, Brar and Minocha 1982). In grass pea, multiple interchanges have been detected among different (Talukdar, 2009). Translocation heterozygosity, with the potential for creating and conserving specific gene blend is generally recognized by the abridged reproductive capacity and the presence of multivalent during reduction division (Sharma and Gohil 2011) The fertility of an interchanged heterozygote depends on the segregation pattern at meiosis I (Adjacent and alternate). In the absence of crossing over in the balanced chromosome amalgamations, adjacent positioning results in unbalanced combinations (Sybenga 1967). As chromosomes are the vehicle to carry genes so any irregularity in their heredity leads to change in the genetic traits. However, this irregularity can originate novelty among traits as the outcome of alteration in the genetic constituent. Chromosome engineering through translocation have evidenced to be the most effectual tool by various researchers to familiarize desirable traits in wheat (Driscroll 1965). Induced mutagenesis has great potential as a complimentary approach in genetic improvement of crop (Sikder *et al.* 2015). For creating genetic variability physical mutagens were used for many years as they causes deletion and occasionally chromosome reconstitution (Priyanka *et al.* 2019) Mutation breeding employing gamma irradiation was initiated to explore its potential for genetic upgradation as well as to elucidate the feasibility of developing translocation and multiple interchange stock, which has tremendous value in linkage analysis (Micke *et al.* 1985).

Artemisia annua L.(Family- Asteraceae) commonly known as sweet wormwood is an annual herbaceous plant. The leaves of *Artemisia* are storage of sesquiterpene, artemisinin which are useful to cure malaria and also effective against chronic bladder infections. The leaves are antiseptic, antiperiodic, digestive and febrifuge. Artemisinin in form of artemisinin-based combination therapy (ACT) is recommended for treatment of malaria caused by *plasmodium* sp. (WHO, 2006). The present study is an attempt to understand the genetic behavior of induced translocation heterozygotes through the male meiotic course including microsporogenesis and pollen fertility which can be utilized for plant breeding program since our knowledge on these facets in case of *Artemisia* is still very limited.

MATERIALS AND METHODS

Procurement of seeds- Fresh and healthy seeds of *Artemisia annua* L. variety EC-415012 were obtained from NBPGR, Bhowali, Nainital India. The 10-10 seeds were kept in three polythene bags and then treated with different doses of gamma rays 100Gy, 200Gy, 300Gy respectively with CO^{60} source at the dose rate of 7.247K Gy/h inside gamma chamber at NBRI, Lucknow. Irradiated seeds were sown in pots along with their respective controls.

Slide preparation for meiotic studies- Young capitulas were fixed in freshly prepared Carnoy's fixative (Alcohol: GAA 3:1) and after 24hrs transferred in 90% alcohol. Slides were prepared by anther squash technique using 2% acetocarmine. Slides were analyzed and suitable cells were photographed under a Nikon photomicroscope. Various rings, chains, and multiple chromosomal associations at metaphase I were examined and recorded. Pollen fertility was also observed using acetocarmine stain. The undersized and unstained pollens were considered as sterile pollen while stained and fully developed pollen grains were considered fertile one. For statistical analysis SPSS 16.0 software used.

RESULTS AND DISCUSSION

Studies on male meiotic course including microsporogenesis and pollen fertility have been made in *Artemisia annua* L. Regular meiosis were found in control plants. The control plants exhibits 9 bivalents at metaphase I (n=9) and 9:9 separations at anaphase I during meiotic division. Treated plants divulges 90-60% survival percentage and among the population single candidate plant as translocation heterozygote in 100Gy set was screen out on the basis of morphological and cytological observation *viz.* reduced seed number, weaker stem and breakage and reunion of non-homologous chromosomes compared to control plant. Depending upon the number of chaismata, the quadrivalents or bivalents in the PMCs were reported at metaphase I stages of meiosis in the form of rings and chain configuration in induced translocation heterozygote. During the cytological diagnosis of induced translocation heterozygotes, a total of 198 PMCs from the single plant were scored at metaphase I. Major chromosomal associations at metaphase I have been shown in **Table 1.**

Fig.1c-i ,shows erratic variation in meiotic chromosome arrangements by forming rings or chains. The ring bivalents are of two types i.e. adjacent (forming simple loop) and alternate (forming an 8shaped bi rings) (**Fig. e,f,g,i**) while chains were divided into an adjacent and zig zag pattern on the basis of their arrangements in side by side or zig zag pattern (**Fig. c,d,h**). Sybenga (1967) classified diagrammatically, these four types of interchange patterns into two types of orientations. According to him, open ring and adjacent associated chromosomes are resulted from adjacent orientation while zig-zag and bi-rings were formed due to alternate orientation. A comprehensible predominance of rings (58.27%) over the chains were perceived, in which open rings and bi-rings

Table 1. Configurations at metaphase I of translocation heterozygote

Associations	2n	No. of PMCs	Frequency (%)
9II	18	20	10.10
1VIII +5II	8+10	32	16.16
1VIII +1IV+1II+4I	8+4+2+4	7	3.53
1VI +6II	6+12	19	9.59
2VI +4I+1II	12+4+2	17	8.58
1VI +2II+2IV	6+4+8	24	12.12
1VI +2IV+1II+2I	6+8+2+2	27	13.63
2VI+1IV+ 1II	12+4+2	7	3.53
1IV +7II	4+14	11	5.55
4IV+1II	16+2	21	10.60
8II+2I	16+2	13	6.56
Total		**198**	

were approximately 38.11% and 20.16%, respectively **(Table 2**). Overall chain configuration was found to be 41.72%; though the occurrence of adjacently oriented chain showed prevalence (30.04%) over the alternate chains (11.68%) (**Table 2**.). Thus, result elucidate that total adjacent orientation was 68.15% and alternate orientation was recorded 31.84% (**Table 2)**. During cytological analysis of induced translocation heterozygote, out of

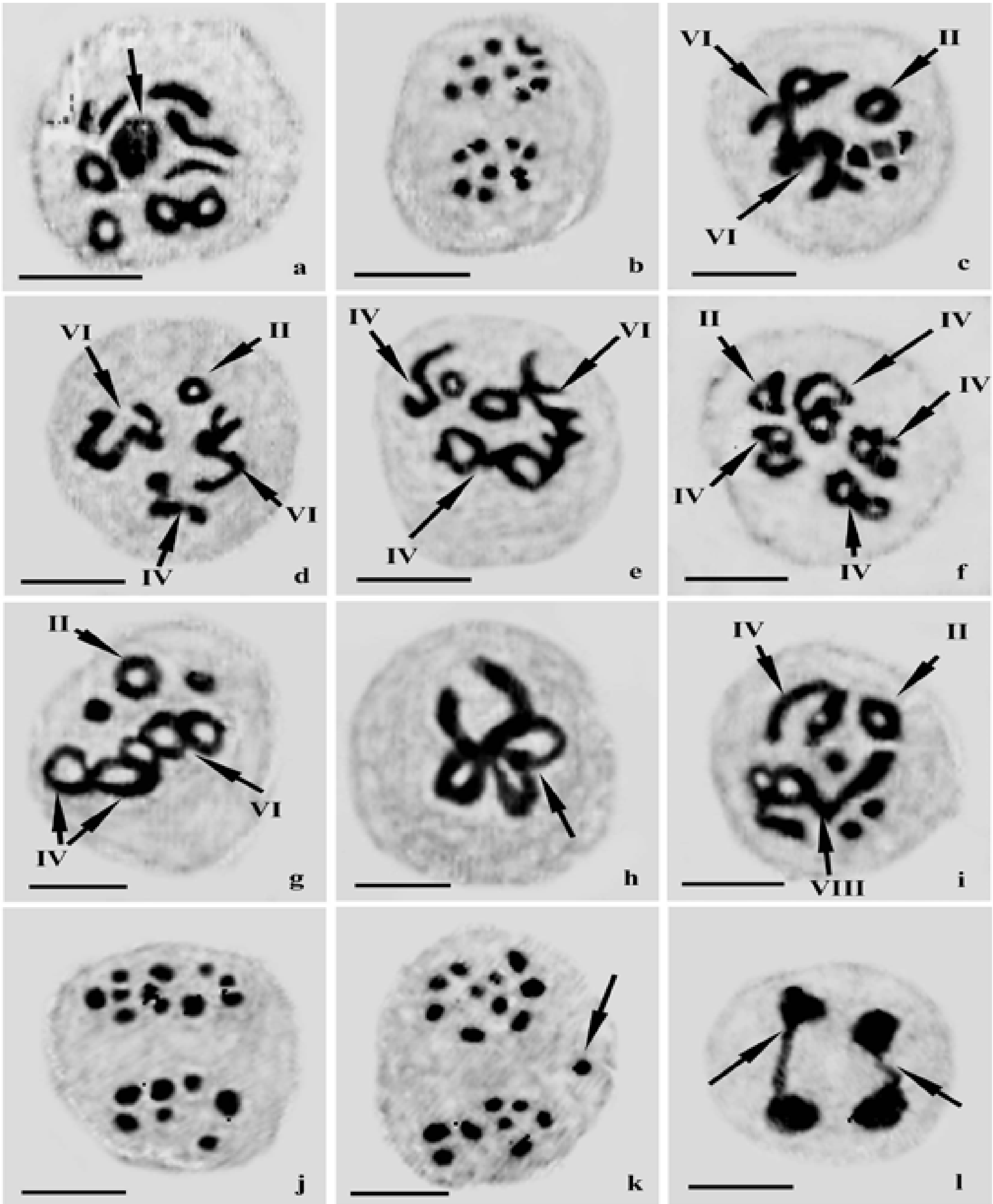

Fig. 1. Meiotic stages in PMCs of *Artemisia annua* L.

a. Diakinesis (9 bivalents with nucleolus), **b.** Normal anaphase (**9:9**), **c-i.** Chromosomal configurations at metaphase I of translocation heterozygote (Arrows show multivalents) **c.** $4_{I}+1_{II}+2_{VI}$ (Knot shaped hexavalent),**d.** $1_{II}+1_{IV}+2_{VI}$ (1W shaped hexavalent chain, 1 γ shaped hexavalent), **e.** $2_{II}+2_{IV}+1_{VI}$ (large 8 shape bi-ring), **f.** 4_{IV}(8 shaped tetravalent)$+1_{II}$, **g.** $2_{IV}+1_{VI}+1_{II}+2_{I}$, **h.** Association of multivalent, **i.** 1_{II} (ring) $+1_{IV}+4_{I}+1_{VIII}$chain, **j.** Unequal separation (**11:7**) at anaphase I, **k.** One laggard at anaphase I, **l.** Bridge formation on both the poles at anaphase II. [**X= 10μm**] a total 198 PMC's, 21 PMC's depicted four tetravalents and one bivalent (**Fig. 1f**) while 27 PMC's showed two tetravalents, one hexavalent, two bivalents and two univalents (**Fig. 1g**). Amidst all maximum number i.e. 32 PMC's deciphers five bivalents and two tetravalents. Besides octavalent and hexavalent along with bivalents, trivalents, pentavalents, nonavalents and decavalents were also observed. In a large percentage of PMC's, chromosomes could separate normally, however some unequal separations were also screened out at frequency of 10.53% (11:7) and 9.21%(6:12) respectively (**Table 3**). **Table 3** gives an account of anaphasic abnormalities such as laggards (**Fig. 1k**) and bridges (**Fig. 1l**). Pollen fertility was observed high in case of control, ensuing to the presence of multivalents and non-synchronous behavior of some bivalents in disjunction it was reduced to just 38.26% in translocated heterozygote. Apparently, increased meiotic disturbance caused by the involvement of more number of different non-homologous chromosomes through irregular segregation in a single plant. This has led to a higher pollen sterility and parallel reduction in seed production of progeny. The unbalanced gametes that lack pollen critical genes generates pollen lethality for isolated translocation (Clark and Krysan 2010). But according to Bala *et al* (2016), Structural heterozygotes which generally show a low fertility and seed setting, demonstrate more than 80% pollen fertility in nine species from district Kangra, Himachal Pradesh, thus specify multivalent formation with alternate disjunction.

Table 2.Chromosomal associations and pollen fertility (%) of translocation heterozygote

Dose	Rings(%)		Chains(%)		Adjacent orientation (%)	Alternate orientation (%)	Pollen fertility (%)
	Open	Bi-ring (8 shaped)	Adjacent	Zig-zag			
Control		66.67	33.33				96.32±0.61
100 Gy	38.11	20.16	30.04	11.68	68.15	31.84	38.26±0.20

Table 3. Frequency of different types of segregations of chromosome at anaphase I of the translocation heterozygote

Segregations	No. of PMCs	Frequency (%)
9:9	33	21.71
11:7	16	10.53
6:12	14	9.21
9:1L:8	41	26.97
9:2L:7	29	19.07
Bridges	19	12.50
Total	**152**	

More than two chromosomal associations in a diploid heterozygote might indicate that at least partial homology of chromosomes extends to some non-homologous pairs, which is achievable either due to hybrid nature of the taxon or heterozygosity for translocation (Singhal 1982). Elevated frequency of ring quadrivalents at metaphase I suggests that the translocation had happened between two comparatively large chromosomes (Soriano 1957). If both pieces of chromosomes are short and chiasmata are not formed in all the arms, chains in place of rings are formed (Burnham 1956). In translocation heterozygote ring and chain association of chromosomes are interrelated with pollen sterility due to non-disjunction of chromosomal segments. Fertility is positively correlated with the percentage of alternate orientation of translocation multivalent (van Heemert and Wijnands-stäb 1975). Ghosh and Datta (2006) reported that the predominant occurrence of adjacent orientation in quadrivalents in heterozygotes are expected to induce high frequency of non-viable male gametes following the duplication and deficiencies of genes. Frequency of univalent was also observed in some PMC's which might be formed by the exchange of interstitial chromosome translocation which does not involve the end. Different chromosomal associations at metaphase I specify the occurrence of inversions. Due to duplication and deficiencies spore of the heterozygote generates non-viable gametes. As according to Sax and Anderson (1932) if an adjacent chromosome moves to the same pole, the produced microspores should be sterile due to deficit of chromosome segment; but if adjacent chromosomes pass to the opposite poles, each microspore should have entire complement and would be capable of further development. In many plant species, individuals with translocations are usually semi-sterile and 50% of the meiocytes display the alternate configuration of the interchange complex at M-I

(Ghaffari *et al.* 2009). Induction of translocation heterozygotes through gamma rays is known in many plant species, *e.g. Pennisetum typhoides* (Pantulu 1967), *Vicia faba* (Sjodin 1971), poppy (Kumar and Naseem 2012) *etc*. The actual cause for induction of translocation by gamma rays is yet unknown, but perhaps gamma radiation ionizes the DNA directly or by reacting with the cell water content and producing free radicals that induce translocation in *Artemisia annua* L. The application of gamma rays might be the probable cause of physiological imbalance in the cells, producing some chemical substances that disrupt the synthesis of DNA in the cells following breakage and restitution of the broken segments, thus resulting in chromosome associations (Basi *et al.* 2006).

The occurrence of anaphasic anomalies like laggards, bridges and unequal separations can be explained to be due to the inability of the multivalent to separate properly (Kumar and Singh 2003). Adjacent orientations were mainly responsible for the formation of laggards because here there are always possibilities of unequal and delayed separation (Kumar and Naseem 2012). Bridges might be formed due to delayed chiasma termilnalization. Gupta *et al*. (2010) reported the structural heterozygosity in *Artemisia parviflora*. Similarly, Malik *et al.* (2010) also reported this phenomenon in *Artemisia absinthium.* But translocation induced through gamma irradiation is reported for the first time in *Artemisia annua* L., that can be utilized for identifying the genetic and linkage correlation.

Heterozygotes are the only source of genetic variations in inbreeding practices; therefore have great importance (Kumar and Bhardwaj 2019). Alteration of gene or a set of genes responsible for pairing might have been affected due to translocation give rise to univalents (Sinha and Acharia 1974). Univalents in succeeding stages of meiosis form aneuploid gametes may lead to the formation of various aneuploid stock (Wani and Bhat 2017).

So, from the aforesaid investigation, It can be concluded that translocation and other meiotic irregularities are the uttermost reason for the incidence of low fertility in plants raised from gamma rays. It's necessity to study that this sterility passes to the further generation or not. These behavioral properties of chromosome involved in translocation may be used for their proficient exploitation in genetics and plant breeding.

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