Meiotic Investigation in Reciprocal Translocation Heterozygotes of *Commelina benghalensis* L. (Commelinaceae) Induced by Gamma Irradiations

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Summary *Commelina benghalensis* L. is a diploid plant with $2n=2x=22$ median size chromosomes. Gamma irradiations (10, 15, 20, 25 kR) to *C. benghalensis* seeds induced 14 translocation heterozygotes. The translocation heterozygotes exhibited the formation of either a ring or a chain of four chromosomes in PMCs (ranged from 79.84 to 100%). The translocation lines showed the preponderance of ring quadrivalents as compared to chain quadrivalents. After comparative analysis, the quadrivalents frequently displayed alternate disjunction (52.91%) than adjacent disjunction (47.09%). At anaphase I/II and telophase I/II, the translocation heterozygotes showed the presence of lagging chromosomes and chromatin bridges. Micronuclei were sometimes formed in tetrads. Heterozygous plants showed high pollen sterility (ranged from 21.30 to 81.82%) due to the presence of adjacent orientation in quadrivalents and the cumulative effect of other meiotic irregularities. Also, pollen sterility showed a positive and significant correlation ($r^2=0.7285$) with adjacent segregation of quadrivalents (ranged from 20.0 to 74.22%) in translocation heterozygotes.

Keywords *Commelina benghalensis*, Gamma irradiation, Meiosis, Quadrivalent, Translocation heterozygote.

Commelina benghalensis L., the family Commelinaceae, is a diploid species with $2n=2x=22$ median size chromosomes. The plant is very beneficial from the medicinal point of view due to some very active compounds. Numerous compounds have been identified from the vegetative and flower parts including noctacanol, *n*-triacotanol, stigma-sterol, campesterol and hydrocyanic acid (Jayvir *et al.* 2002). Phytochemical screening also revealed the presence of many secondary metabolites like phlorotannins, carbohydrates, tannins, glycosides, volatile oils, resins, balsams, flavonoids, and saponins (Jemilat *et al.* 2010). Presence of flavonoids, for example, indicates the plant might have an antioxidant, anti-allergic, anti-inflammatory, anti-microbial or anti-cancer activity (Kunle and Egharevba 2009). Also, *C. benghalensis* has an algicidal action that proves the folkloric use in pain management (Hasan *et al.* 2010). Furthermore, recent studies show that *C. benghalensis* has many essential functional components for antibacterial activity (Khan *et al.* 2011).

Genetic variation is very important for improvement in the important features which are of medicinal value. Induced mutagenesis is an efficient method for the induction of morphological and genetic variability's in plants. Gamma irradiations have been efficiently utilized

from the past few decades in creating rapid genetic variability in some important traits. The gamma rays normally induce DNA lesions after producing free radicals which subsequently damages or modifies important gene products of plant cells (Khah and Verma 2017). Besides gross chromosomal aberrations, mutagens also cause point mutations in the individual genes leading to an increase in genetic variability in the segregating generations (Khah *et al.* 2018).

Reciprocal translocations arise by the exchange of broken segments of non-homologous chromosomes. Reciprocal translocations are usually identified by the presence of characteristic multivalent associations at metaphase I followed by partial pollen and ovule abortion (Khah and Verma 2020). Along with different aneuploids and other structural chromosomal aberrations, reciprocal translocation served as excellent cytogenetic tools for the identification and mapping of different linkage groups in plants (Sybenga 1996). It has also been involved successfully in transferring desirable traits (Sears 1956, Gustafsson 1965) and to generate different trisomics in a number of crop plants (Ashraf and Bassett 1987, Lakshmi and Nalini 1989, Talukdar 2009). The present study has been undertaken 1) to identify the translocation heterozygotes among the mutagenic populations 2) to study the meiotic behavior of these translocation heterozygotes by analyzing the configuration of interchange complexes and 3) to investigate the

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segregation pattern of chromosome complexes followed by their impact on pollen fertility.

Materials and methods

Plant material and gamma ray treatment

Healthy and fresh seeds of *C. benghalensis* L. were collected from the experimental field of School of Studies in Botany, Vikram University Ujjain, Madhya Pradesh, India. In total, 1000 seeds were subjected to various doses of gamma rays at Bhabha Atomic Research Center, Mumbai with the treatment doses 5, 10, 15, 20, 25, 30, 35, 40, and 50 kR. For each control and treatment dose, 100 seeds were then sown in separate pots with 10 seeds in each pot.

Cytological preparation and meiotic analysis

For meiotic studies, young floral buds were fixed in

freshly prepared acetic acid–ethanol (1:3) fixative for at least 24 h and stored in a refrigerator until use. Slides were prepared using anther squash technique with 2% iron acetocarmine. Temporary slides were analyzed and suitable cells were photographed in an Olympus microscope. Observations were recorded on chromosome configurations at diakinesis and metaphase I with interchanged complex as a ring or chain associations. The interchanged complexes were assigned as alternate or adjacent segregations based on proposed configurations of Sybenga (1968). In alternate segregation, ring interchange complex appears like the symbol “8”, whereas a chain multiplex takes a zigzag orientation. However, open ring and linear chain configurations of multivalents are characteristic of adjacent disjunction. Pollen fertility was also estimated using 2% acetocarmine. Fertile pollen grains were recorded with stained nuclei whereas undersized and unstained pollen grains without nuclei

Fig. 1. Meiotic stages in control and translocation heterozygotes. a) Normal diakinesis with 11 bivalents ($2n=22$). b) Anaphase I with normal separation (11:11). c–d) One open ring quadrivalent with normal 9_{II} . e) One 8-shaped quadrivalent with normal 9_{II} . f–g) One chain quadrivalent with normal 9_{II} . h) One chain quadrivalent (zig-zag shape) with normal 9_{II} . i) Chromatin bridge at anaphase I. j) Triad. k) Tetrad with micronuclei. l) Fertile (stained) and sterile (unstained) pollens. Scale bar=10 μ m.

were considered sterile.

Results

Meiosis was found normal in the PMCs of control plants which showed regular 11 bivalents at diakinesis and metaphase I (Fig. 1a). At anaphase I, chromosomes showed normal separation (Fig. 1b) followed by normal cytokinesis and regular formation of tetrads. Pollen fertility was normal in control populations (93.42%).

In gamma irradiation populations, 14 plants (designated from CT-1 to CT-14) were found heterozygous for reciprocal translocations. Cytogenetic characterization of these plants showed ring and chain configurations of four chromosomes at diakinesis and metaphase I (Fig. 1c–h). The irradiation dose and percentage of PMCs showing the translocation complexes are shown in Table 1. The frequency of PMCs with interchange complex ranged from 79.84 to 100% (Table 1). The translocation heterozygotes, CT-1, CT-2, CT-4, CT-7-10, and CT-12-14, were characterized by the presence of $1_{IV}+9_{II}$

in all PMCs at metaphase I. However, the translocation heterozygotes, CT-3, CT-5, CT-6 and CT-11 showed the occurrence of interchange complexes in 86.99, 79.84, 87.84 and 91.14% of cells, respectively. Out of the total 14 translocation heterozygotes, six plants were isolated from 25kR M1 population while the four plants from 15kR populations. The other interchange heterozygotes were induced by 20kR (two plants) and 10kR irradiation doses (two plants).

Orientation behavior (alternate and adjacent) of interchange complexes was also studied by examining the meiotic configuration of translocation multiples at metaphase I (Table 2). A clear preponderance of rings (76.07%) over the chains was observed, in which the mean percentage of open rings and bi-rings was approximately 32.85% (SD=±13.60) and 43.22% (S=±16.11), respectively (Table 2). The overall chain configuration was found to be 23.93%; though the occurrence of adjacently oriented chains showed prevalence (Mean=14.24; SD=±10.82) over the alternate chains (Mean=9.69%; SD=±7.51). Thus, the overall percentage mean of the

Table 1. Irradiation dose, percentage of PMCs at metaphase I with translocation complex and pollen sterility of translocation heterozygotes of *C. benghalensis*.

Interchange heterozygote	Irradiation dose (kR)	PMCs analyzed	PMCs showing translocation complex	PMCs showing translocation complex (%)	Pollen sterility (%)
CT-1	10	142	142	100	37.98
CT-2	10	113	113	100	26.53
CT-3	15	146	127	86.99	46.75
CT-4	15	108	108	100	24.48
CT-5	15	129	103	79.84	55.20
CT-6	15	148	130	87.84	21.30
CT-7	20	104	104	100	32.58
CT-8	20	162	162	100	81.82
CT-9	25	122	122	100	34.19
CT-10	25	137	137	100	54.92
CT-11	25	158	144	91.14	47.76
CT-12	25	204	204	100	45.27
CT-13	25	119	119	100	51.85
CT-14	25	173	173	100	28.99

Table 2. Chromosomal configuration and disjunction at metaphase I in the translocation heterozygotes of *C. benghalensis*.

Interchange heterozygote	Quadrivalents analyzed	Ring of 4 chromosomes (%)		Chain of 4 chromosomes (%)		Alternate disjunction (%)	Adjacent disjunction (%)
		O-shaped	8-shaped	C-shaped	Z-shaped		
CT-1	129	33.33	52.71	9.30	4.65	57.36	42.64
CT-2	97	7.22	50.52	13.40	28.87	79.38	20.62
CT-3	127	41.73	30.71	21.26	6.30	37.01	62.99
CT-4	99	15.15	72.73	—	12.12	84.85	15.15
CT-5	103	32.04	20.39	41.75	5.83	26.21	73.79
CT-6	130	20.00	63.85	—	16.15	80.00	20.00
CT-7	91	47.25	50.55	2.20	—	50.55	49.45
CT-8	128	49.22	16.41	25.00	9.38	25.78	74.22
CT-9	99	26.26	48.48	8.08	17.17	65.66	34.34
CT-10	119	49.58	36.13	14.29	—	36.13	63.87
CT-11	144	18.75	52.78	19.44	9.03	61.81	38.19
CT-12	176	50.00	36.36	10.23	3.41	39.77	60.23
CT-13	110	40.91	21.82	21.82	15.45	37.27	62.73
CT-14	151	28.48	51.66	12.58	7.28	58.94	41.06

Table 3. Frequency of various meiotic irregularities at anaphase I/II and telophase I/II in translocation heterozygotes of *C. benghalensis*.

Interchange heterozygote	Total cells analyzed	Total abnormal cells	Laggards (%)	Bridges (%)	Micronuclei (%)	Abnormal tetrads (%)
CT-1	113	26	7.9	6.2	3.5	5.3
CT-2	142	38	10.6	9.1	4.9	2.1
CT-3	107	13	3.7	2.8	3.7	1.9
CT-4	119	23	6.7	5.0	4.2	3.4
CT-5	105	19	5.7	4.8	3.8	3.8
CT-6	103	18	5.8	3.9	2.9	4.8
CT-7	148	43	12.8	8.1	4.0	4.0
CT-8	137	31	9.5	8.0	2.9	2.2
CT-9	122	24	5.7	7.4	2.4	4.1
CT-10	129	38	10.8	8.5	6.2	3.9
CT-11	118	24	9.3	5.9	1.7	3.4
CT-12	107	18	4.7	6.5	3.7	1.9
CT-13	143	37	9.8	8.4	4.9	2.8
CT-14	153	44	13.7	9.1	2.0	3.9

incidence of adjacent and alternate orientations was found as 47.09% and 52.91%, respectively. The meiotic disjunction of interchange chromosomes is very critical as it regulates the fate of the cell. In this study, out of total 1703 quadrivalents analyzed, 889 (52.20%) showed alternate type of segregation and 814 (47.80%) showed adjacent segregation. Among different chromosomal associations, ring quadrivalents mostly tend to orient in alternate types rather than adjacent types. Among 1703 quadrivalents, 1303 (76.51%) showed ring configuration at metaphase in which 732 quadrivalents (56.18%) displayed alternate disjunction and 571 quadrivalents (43.82%) possessed adjacent segregation.

The translocation heterozygotes displayed various meiotic anomalies at anaphase I/II and telophase I/II (Table 3, Fig. 1i–k). The anomalies included lagging chromosomes (mean=8.34; range=3.7–13.7%) and chromatin bridges (mean=6.69; range=3.9–9.1%) and. The lagging chromosomes were found in the form of univalents or as whole bivalents. Abnormal cytokinesis led to the formation of dyads, triads, pentads, and hexads in translocation heterozygotes (Fig. 1j). The tetrads also exhibited the presence of micronuclei (Fig. 1k) among interchange heterozygotes. The number of micronuclei within tetrads ranged from one to four, but the maximum exhibited one or two micronuclei. The pollen fertility was also reduced in translocation heterozygotes (Table 1) which ranged from 21.30 to 81.82% in CT-12 and CT-7, respectively. The translocation heterozygotes showed stunted growth with weak stem and high sterility. Most of the heterozygotes displayed no seed set.

Discussion

Interchanges, involving the exchange of chromosome segments, have massive importance as they signify a model of surveying genetic and cytological changes, and provide novel gene combinations by breaking undesirable associations. Gamma irradiations have been successfully

employed for creating chromosomal interchanges in a number of plant species (Verma and Raina 1990, Verma and Goyal 2012, Verma and Shrivastava 2014, Khah and Verma 2017, 2020). These interchange heterozygotes are a significant tool for chromosome manipulation and sustainable interchange heterozygotes have great significance in breeding programs of the plant.

In the present study, 14 translocation heterozygotes were identified by the presence of ring and chain configurations of four chromosomes at diakinesis and metaphase I. In all translocation heterozygotes observed, the number of ring quadrivalents was higher than that of the chain quadrivalents. Various factors that affect the meiotic configuration of interchange multiples include the morphology of translocated chromosomes, the position of the translocated part, position and number of the chiasma, degree of chiasma terminalization, and arm ratio (Sybenga 1968, Verma and Raina 1990, Khah and Verma 2020). Interchange chromosomes with break sites near centromere and more or less equal in length causes a high incidence of ring configuration. However, shorter the interchanged segments the more likely it fails to pair with the homologous segment, and therefore, chain formation takes place. Also, chiasma failure may produce an open-chain association of a multivalent instead of a ring. In the present study, the high prevalence of ring quadrivalents in translocation heterozygotes seems to be due to a larger length of interchanged segments as well as stable chiasma associations in all the chromosome arms.

The translocation heterozygotes also showed a reduction in pollen fertility. The orientation of translocation multiples at metaphase I has a great bearing on the fertility of interchange heterozygote (Verma and Raina 1990). Alternate I and II segregation give rise to fertile gametes while adjacent I and II give rise to nonviable duplication and deficiency gametes. When these two types of segregations are of equal frequency, the result is semi-sterility. The difficulty of the quadrivalents to segregate accurately at anaphase I/II or telophase I/II

Fig. 2. Correlation between adjacent disjunction of interchange multiples and sterility of pollen grains.

creates various abnormalities which include laggards, bridges, micronuclei and others. The cumulative effects of these abnormalities also contribute to pollen sterility later in translocation heterozygotes. A high degree of pollen sterility observed in both semi-sterile translocation heterozygotes can be attributed not only to anaphase and telophase abnormalities but also to adjacent types of disjunctions ($r^2=0.7285$, Fig. 2). If such translocation lines are established by removing their sterility barrier, novel gene combinations are expected to be induced through trisomics isolation which could be beneficial in future cytogenetic programs.

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