Gamma Radiation as a Mutagen in *Chrysanthemum carinatum*: Discovery of Translocation Heterozygotes

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Abstract

This study focuses on gamma radiation-induced translocation heterozygotes in *Chrysanthemum carinatum*, aiming to explore the role of mutagenesis in plant breeding and cytogenetic research. The objective was to examine the potential of gamma radiation in inducing chromosomal changes, specifically translocations, in ornamental plants, which can lead to phenotypic variations. The research was conducted at the Botanical Garden, University, School of Basic and Applied Sciences, Sangam University, Bhilwara, where gamma-irradiated plants were cultivated to assess the effects of radiation on genetic diversity and ornamental traits.

To study the chromosomal behavior, flower buds were collected from the irradiated plants for analysis during meiosis. This process enabled the identification of translocation heterozygotes, and five such heterozygotes were successfully observed. The results indicate that gamma radiation can effectively induce chromosomal translocations, contributing to genetic variability and potential improvements in morphological traits. The findings align with previous research on the use of gamma irradiation in various plants (Banerji & Datta, 2002; Azad et al., 2022) and demonstrate its effectiveness in advancing plant breeding, enhancing traits, and improving stress tolerance (Jiang et al., 2022; Rieger & Michaelis, 1962). Additionally, the study represents a unique contribution to the field by conducting research under the arid and semi-arid climatic conditions of Rajasthan, an area not previously explored for plant mutagenesis. This work demonstrates the potential for using gamma radiation to enhance genetic diversity and improve ornamental traits, especially in regions with challenging environmental conditions.

Key words; *Chrysanthemum carinatum*, Translocation heterozygotes, Gamma radiation, Mutagenesis.

Chrysanthemum carinatum, a prominent ornamental plant, is highly valued worldwide for its vibrant, colorful blooms and versatile uses. Known for its daisy-like flower shapes and a wide spectrum of colors, including yellow, white, purple, and red, chrysanthemums are a staple in floral arrangements, bouquets, and gifts. Beyond their ornamental appeal, several chrysanthemum species are also recognized for their medicinal properties and are used in herbal teas and dietary supplements.This tropical plant is part of the Asteraceae family, one of the largest and most diverse families of flowering plants, with over 1,620 genera and 23,600 species globally, accounting for around 10% of all flowering plant species (Funk et al., 2009). In India, the family includes 167 genera and approximately 900 species, with the Kashmir Himalayas being home to 260 species known for their medicinal and aromatic benefits (Hajra et al., 1995; Dar & Khuroo, 2013). Chrysanthemums have a long cultivation history, dating back to the 15th century BCE in China, where they were initially grown as flowering herbs.

Mutations, defined as inheritable changes in genetic material, are fundamental to the evolution and diversification of living organisms. These changes can occur naturally, referred to as spontaneous mutations, or can be induced artificially using external agents like chemical mutagens and ionizing radiation. Spontaneous mutations arise independently in nature, significantly contributing to genetic diversity. In contrast, induced mutations are deliberately introduced to manipulate genetic material, allowing researchers to investigate genetic mechanisms and develop novel traits. Among the tools for inducing mutations, ionizing radiations have gained prominence for their ability to cause chromosomal aberrations and structural alterations, making them invaluable for geneticists and cytologists.

Ionizing radiation, particularly gamma rays, is highly effective in inducing chromosomal mutations. These mutations result in significant changes in the structure and function of genetic material, providing insights into chromosomal behavior while offering practical applications in plant breeding and genetic research. Mutation induction techniques have been extensively utilized to develop new traits, enhance crop characteristics, and increase genetic variability. Through manipulation of the genetic makeup, these methods address critical agricultural challenges, including improved yield, resilience to environmental stresses, and adaptability to changing climatic conditions.

In this study, gamma irradiation was used to induce mutations in *Chrysanthemum carinatum*, leading to the isolation of five translocation heterozygotes. These heterozygotes exhibit unique chromosomal configurations resulting from the rearrangement of segments from non-homologous chromosomes. Such chromosomal alterations are pivotal for advancing our understanding of genetic mechanisms and exploring their applications in breeding programs. Recent findings, such as those by Sybenga et al. (2012), have demonstrated the role of trisomy in enhancing interstitial crossing-over in translocation heterozygotes of *Secale*, further emphasizing the broader importance of induced mutations in cytogenetic research.

Gamma irradiation has established itself as a versatile and effective method for inducing genetic variability through chromosomal aberrations and mutations. This technique plays a crucial role in understanding chromosomal behavior, identifying structural alterations, and improving genetic traits in a wide range of plant species. For example, Azad et al. (2022) reported gamma-induced chromosomal aberrations in meiotic cells of *Triticum aestivum*, underscoring its value in plant breeding and cytogenetic studies.

Similarly, in ornamental plants like chrysanthemums, Banerji and Datta (2002) successfully induced mutations that altered flower head shapes, demonstrating the potential of gamma irradiation for aesthetic and commercial plant improvement. Additionally, Haspolat (2023) emphasized the role of mutagenesis in enhancing genetic diversity in chrysanthemums, reinforcing the importance of gamma irradiation in modern plant improvement.

The primary objective of this study is to explore the effects of gamma irradiation on inducing chromosomal translocations in *Chrysanthemum carinatum* and to isolate and characterize translocation heterozygotes. This research aims to document chromosomal rearrangements, which are crucial for plant breeding and cytogenetic studies. Similar studies have successfully induced mutations in species like *Vicia faba* (Rieger and Michaelis, 1962; Schubert et al., 1982) and *Chrysanthemum morifolium* (Wang et al., 2023), where gamma irradiation led to desirable phenotypic changes in ornamental traits. Additionally, *Helianthus annuus* (Jiang et al., 2022) and *Tagetes erecta* (Lin et al., 2023) have shown the potential of mutagenesis for enhancing stress tolerance and floral traits, underscoring the value of gamma irradiation in Asteraceae species. This study aims to expand on these findings by evaluating translocation heterozygotes in *Chrysanthemum carinatum*, contributing to genetic diversity and breeding advancements.

Materials and Method

Dry seeds (6.4% moisture) of *Chrysanthemum carinatum* were irradiated by 5, 10, 15, 20, and 25 kR doses of gamma rays at the B.A.R.C., Mumbai (Patil et al., 2015). The control and irradiated seeds were sown in pots. For meiotic analysis, young flower buds were fixed in a 1:3 mixture of acetic acid and absolute alcohol for 24 hours, following standard cytogenetic procedures (Sharma & Sharma, 1999). The anthers of appropriate size were squashed in 2% iron-acetocarmine, a commonly used stain for meiotic chromosome studies (Jones, 2008). Photographs were taken using temporary preparations with a Leica photographic microscope (Singh et al., 2010).

Observations and Results

Control plants showed 9 bivalents ($2n=18$) at diakinesis/metaphase I in all the 50 PMCs observed. Anaphase I distribution of chromosomes was normal (9: 9). Pollen stainability was more than 94%.Five translocation heterozygotes were isolated in M_1 generation and were designated as CcT_1, CcT_2, CcT_3, CcT_4 and CcT_5, respectively. One plant of each (CcT_1, CcT_2, CcT_3, CcT_4, CcT_5,) translocation heterozygote was isolated from the population raised from 15 kR, 20 kR, 25 kR and 30kR. Two plants (CcT_1 and CcT_2) were selected from the seeds irradiated with a 15 kR dose.

The study investigated the effects of gamma radiation (5 kR to 30 kR) on meiosis and chromosomal behavior in *Chrysanthemum carinatum*. Observations at diakinesis/metaphase-I recorded 9 bivalents per cell, with 3 associated with the nucleolus. The number of ring bivalents ranged from 5–7, and rod bivalents ranged from 1–3, with averages of 5.20–5.70 and 1.72–3.70, respectively, across different radiation doses. Chiasma frequency per cell declined from 30.75 (control) to 24.04 (20 kR), and the terminalization coefficient decreased from 0.829 (control) to 0.736 (20 kR). By metaphase-I, nucleoli disappeared, and bivalents aligned on the metaphase plate. Chromosome segregation was regular, showing a 9:9 distribution at anaphase-I forming four groups of 9 chromatids at anaphase-II. Gamma radiation also impacted germination and survival. The control group exhibited 100% germination and no abnormalities. Survival rates declined with increasing doses, with a significant drop at 30 kR, where only 45% of seeds survived. (Table 1).

Table-1 Chromosomal associations Chiasma frequency and terminalization observed at different doses of gamma radiation in *Chrysanthemum carinatum* at dikinesis /Metaphase.

	No. of Slides	**Ring**	**Rod**	**Total**	**Chiasma frequency/**	**Chiasma**	**Chiasma**	**Terminalization**
Doses	**Analysed**	**Bivalents**	**Bivalents**	**Bivalents (mean)**	**cell**	**Terminalized**	**Unterminalized**	**Coefficient**
		(mean)	**(mean)**			**(mean)**	**(mean)**	
Control	10	6.83	2.23	9.16	30.75	25.5	4.2	0.829
5kR	10	7.3	1.7	9.1	32.63	29.33	1.3	0.892
10kR	10	5.6	3.33	8.93	28.6	22	1.6	0.769
15kR	10	5.7	3.33	9.03	28.16	21.5	2	0.763
15kR	10	5.4	3.6	9.5	27.09	21	2	0.775
20kR	10	5.2	3.8	9	24.04	17.71	2.66	0.736

Table -2. Chromosomal associations at diakinesis/metaphase-I in induced translocation heterozygotes of *Chrysanthemum carinatum* (CcT_1-CcT_5)

Genotype	**Mutagen**	**No. of**	**Types of associations (%)**							
		cells								
		analysed								
			IIV+7II	**2IV+5II**	**3IV+3II**	**1IV+6II+2I**	**2IV+4II+2I**	**3IV+2II+2I**	**8II+2I**	**9II**
Control	-	50	6	-	-	-	-	-	-	94
CcT_1	15kR	50	33	-	-	8	3	-	5	-

CcT$_2$	15kR	50	30	-	-	10	4	-	8	-
CcT$_3$	20kR	50	32			7	6			
CcT$_4$	25kR	50	8	16	-	-	12	-	-	-
CcT$_5$	30kR	50	-	-	26	-	-	10	12	-

Table -3. Frequency of ring and chain of 4 chromosomes at diakinesis/metaphase I is translocation heterozygotes of *C.carinatum* (CcT$_1$-CcT$_4$)

Genotype	**No. of cells**	**Ring of 4**		**Chain of 4**		**Ring + chain of 4**		**No. of bivalents**
	analysed	**No.**	**Mean**	**No.**	**Mean**	**No.**	**Mean**	
Control	50	4	0.08	-	-	-	-	8.9
CcT$_1$	50	33	0.66	8	0.16	4	0.98	6.9
CcT$_2$	50	34	0.68	6	0.12	8	0.16	6.2
CcT$_3$	50	40	0.8	10	0.2	41	0.82	7.02
CcT$_4$	50	76	1.52	28	0.56	86	1.72	5.1
CcT$_5$	50	87	1.74	52	1.04	160	3.2	4.2

Gamma Radiation-Induced Translocation Heterozygotes in *Chrysanthemum carinatum*

Chromosomal analysis of the M1 population derived from gamma-irradiated seeds identified five distinct translocation heterozygotes (CcT1 to CcT5), characterized by structural chromosomal rearrangements.(Tabl-3). The frequency of translocation heterozygotes varied with the radiation dose, indicating a threshold effect for chromosomal instability.CcT$_1$ (*15 kR*): Quadrivalent formations were observed in 98% of PMCs, with 33 cells showing ring configurations and 8 displaying chains. At diakinesis/metaphase-I, 19 quadrivalents exhibited an '8' shape, and 47 showed open rings. Pollen fertility was reduced to 54%.CcT$_2$ (*15 kR*): Quadrivalents were present in 92% of PMCs, with 34 rings and 6 chains observed. Among these, 24 had an '8' shape configuration and 40 were open rings.(Table-5)

Table-4 The different types of chromosomal associations at anaphase/telophase I/II in induced translocation heterozygotes of *C.carinatum* (CcT$_1$-CcT$_4$)

Genotypes	No. of cells	Diakinesis				Anaphase/Telophase-I		Anaphase/Telophase-II	
	Aalyzed	No. of nucleoli				1 or 2 Leggards	Micronucleoli	1 or 2 Laggards	Micronucleoli
		1	2	3	4				
Control	50	50	-	-	-	-	-	-	-
CcT$_1$	50	14	28	8	7	12	18	4	3
CcT$_2$	50	15	26	6	5	10	12	6	2
CcT$_3$	50	13	32	-	-	14	8	5	6
CcT$_4$	50	12	18	18	2	5	14	6	9
CcT$_5$	50	16	-	29	5	6	8	2	15

Pollen fertility was recorded at 53%.CcT$_3$ (*20 kR*): All PMCs exhibited quadrivalents, with 40 rings and 10 chains identified. At diakinesis/metaphase-I, 22 quadrivalents displayed an '8' shape and 42 open rings. Pollen fertility was lower, at 43%.CcT$_4$ (*25 kR*): Quadrivalents were detected in 76% of PMCs, with 40 rings and 28 chains. Among these, 33.8 exhibited an '8' shape configuration, while 48 formed open rings. Pollen fertility was recorded at 38%.CcT$_5$ (*30 kR*): Quadrivalents were observed in 28% of PMCs, with 86 rings and 52 chains. At diakinesis/metaphase-I, 41.14 quadrivalents displayed an '8' shape, and 39 were open rings. Pollen fertility was the lowest, at 28%.(Table-5)

Table-5 Open ring and 8 shaped quadrivalents and pollen fertility in translocation hetrozygotes.

Genotype	No .of cells Analysed	Quadrivalents		Quadrivalents		Pollen Fertility %
		Open	%	8 Shaped	%	
Control	50	4.5	9		-	94
CcT$_1$	50	23.5	47	9.5	19	54
CcT$_2$	50	20	40	12	24	53
CcT$_3$	50	21	42	11	22	43
CcT$_4$	50	24	48	16.9	33.8	38
CcT$_5$	50	27.5	39	20.57	41.14	28

Across all translocation heterozygotes, lagging chromosomes, micronuclei, and variations in nucleoli numbers were frequent during meiotic divisions. The observed reductions in pollen fertility suggest that gamma radiation-induced chromosomal rearrangements significantly impact reproductive efficiency, offering potential applications in genetic studies and breeding programs.

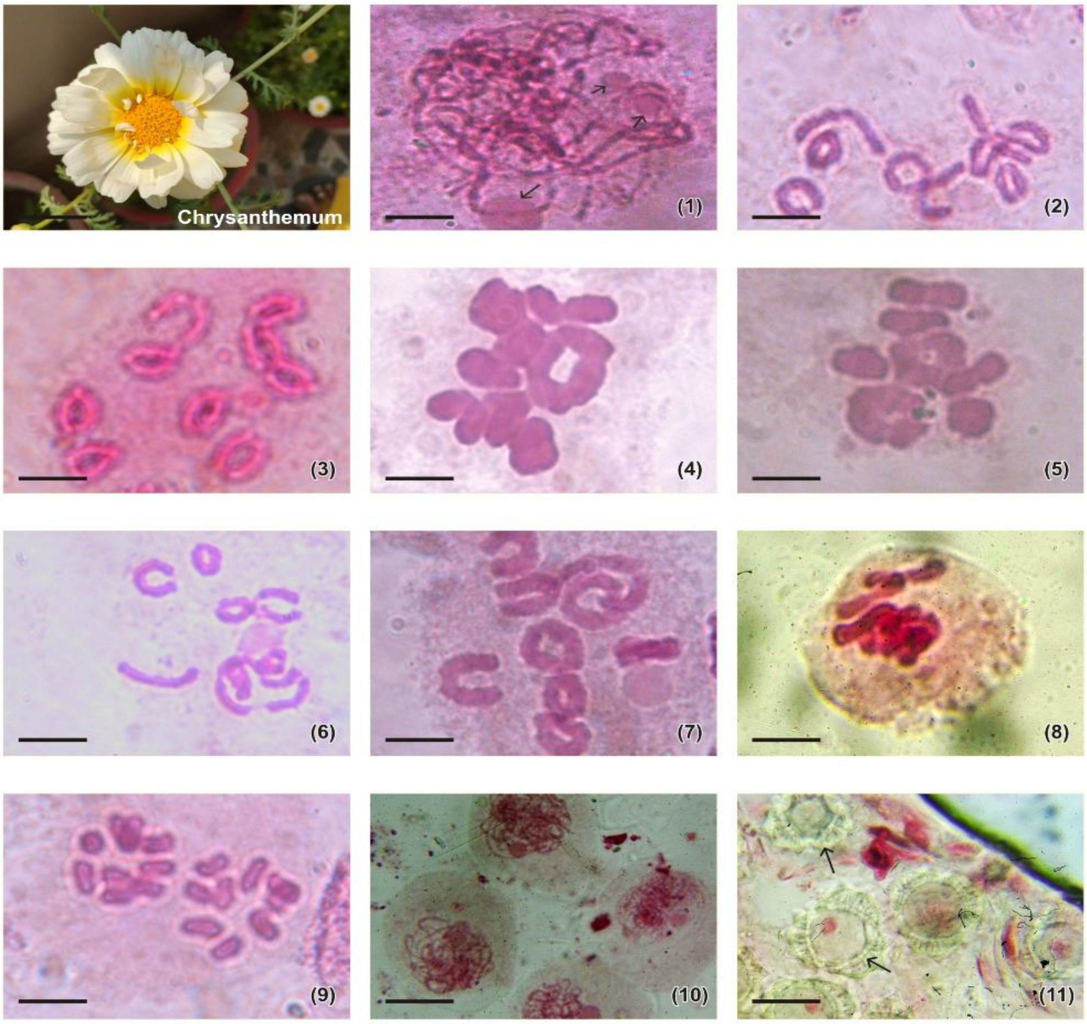

Fig. Plate No.1- **(1)** 3.Supernumerary nucleoli at interphase 30kR. **(2).** 2IV (2 chain) at Diakinesis on 30kR **(3).**1VI chain,5ring II,1rod bivalent &1 nucleolus at dikinesis 20kr. **(4).**1IV ring and 7 II(3ring&4rod) at 15kR **(5).**2IV.& 5II (1 ring & 4 rod)at metaphase I-25kR. **(6)** 1VI Chain &,1IV,3II ring 1II rod dikinesis 25kr **(7)** 9 II (8 ring & 1 rod)at metaphase I control, **(8)** Metaphase I control, **(9)** late anaphase I 20 kr 9_9, **(10)** Interphase at control, **(11)** Image-31_2023-05-11 stained and unstained pollen (Bar = 10μm)

Discussion

Gamma radiation is a well-established mutagenic tool that induces genetic variability in plants by causing chromosomal rearrangements such as translocations. This study on *Chrysanthemum carinatum* further demonstrates the effectiveness of gamma irradiation in generating translocation heterozygotes, which are important for improving ornamental traits in plants. Gamma radiation is known to induce mutations that alter various plant characteristics, such as flower shape, color, and size, which are crucial both aesthetically and commercially, especially in ornamental species like chrysanthemums (Banerji & Datta, 2002; Azad et al., 2022).

The successful induction of translocation heterozygotes in this study aligns with previous research that showed how gamma irradiation can lead to chromosomal alterations and desirable phenotypic changes in other species (Haspolat, 2023; Wang et al., 2023). These findings suggest that gamma radiation is an effective tool for inducing beneficial mutations in ornamental plants, with the potential to enhance traits that have both aesthetic and practical applications.

Translocation heterozygotes, like those created in *Chrysanthemum carinatum*, are valuable not only for their role in enhancing plant traits but also for advancing our understanding of chromosomal behavior. Their induction helps increase genetic diversity, which is vital for breeding resilient and productive plant varieties (Sybenga et al., 2012). This approach also provides insights into structural chromosome alterations, offering new opportunities for targeted breeding programs to improve specific plant traits (Jiang et al., 2022).

Moreover, the creation of translocation heterozygotes in *Chrysanthemum carinatum* highlights the broader potential of gamma irradiation in plant breeding. Similar studies in other species, such as *Helianthus annuus* and *Vicia faba*, demonstrate how gamma radiation can generate desirable mutations that not only enhance aesthetic traits but also improve stress tolerance and overall plant fitness (Jiang et al., 2022; Rieger & Michaelis, 1962; Lin et al., 2023).

The findings of this study further support the application of gamma irradiation as a promising tool in modern plant breeding, particularly for improving ornamental traits, increasing genetic diversity, and enhancing stress tolerance in plants, especially in regions with challenging climatic conditions like Rajasthan.

Conclusion

This research successfully induced translocation heterozygotes in *Chrysanthemum carinatum* through gamma irradiation, confirming the potential of this mutagenesis technique to enhance ornamental traits and promote genetic diversity. The results align with previous studies and contribute to the growing body of knowledge on the use of gamma radiation in plant breeding.

The induction of translocation heterozygotes offers valuable insights into chromosomal behavior and provides a foundation for future studies on improving plant traits and resilience. This study is significant not only for its contributions to ornamental plant breeding but also for its pioneering work in Rajasthan's unique climatic conditions, demonstrating the broader potential of gamma irradiation in plant breeding.

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