

## Genetic repairing through storage of gamma irradiated seeds in inbred maize (*Zea mays* L.)

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**Abstract:** Gamma irradiation can induce beneficial as well as deleterious impacts on chromosome behavior in crop plants. The cytogenetic changes occurring due to the storage of inbred seeds after gamma irradiation in the somatic and gametic cells of *Zea mays* L. were investigated in this study. A wide spectrum of chromosomal anomalies was encountered in somatic and gametic cells of maize that are gamma irradiated, stored (aged), and treated with a combination of both of these treatments. Gamma rays and ageing treatments induced a number of chromosomal anomalies independently, but a combination of the 2 treatments suppressed the frequency of chromosomal anomalies considerably in somatic as well as gametic cells of maize. It is evident from our study that the decrease in mitotic index was inversely proportional to the increase in doses in all the sets of gamma rays and ageing treatments. The conclusion drawn from our study is that individual treatment of gamma rays induced the highest percentage of chromosomal abnormalities, followed by the combination treatment of ageing and gamma rays, and the least number of abnormalities were induced by the individual treatment of ageing. Hence, the combination treatment proved to be better in the mechanism of genetic repairing, which is discussed in the present study.

**Key words:** *Zea mays*, gamma irradiation, storage, mitosis, meiosis, chromosomal anomalies

### Gamma radyasyonuna ve depolamaya maruz bırakılmış mısırlarının genetik tamiri

**Özet:** Gamma radyasyonu faydalı etkilerinin yanısıra bu bitkinin kromozomlarında öldürücü etkiler de yapabilir. Bu çalışmada gamma radyasyonu sonrası depolanan *Zea mays* L. mısırlarında meydana gelen sitogenetik değişiklikler incelenmiştir. Gamma radyasyonuna maruz bırakılmış ve depolanmış somatik ve gametik mısır hücrelerinde kromozom anormallikleri oluşmuştur. Gamma ışınları ve bekletme birbirinden bağımsız olarak bazı kromozom anormalliklerine neden olmuştur. Fakat bu iki muamelenin kombinasyonu hem gametik hem de somatik hücrelerde kromozom anormallik sıklığını baskılamıştır. Bizim çalışmamızda da gözlemlendiği gibi mitotik indekste azalma gamma radyasyonuna maruz kalma ve bekleme dozajlarındaki artışla ters orantılıdır. Sonuç olarak gamma ışınının tek başına uygulanması en yüksek oranda kromozom anormalliğine sebep olurken bunu kombinasyon muamele takip etmiş, ve en az anormallik depolayarak bekletme uygulamasında oluşmuştur. Böylece farklı muamelelerin birlikte uygulanması ile ortaya çıkan genetik tamir bu çalışmada incelenmiştir.

**Anahtar sözcükler:** *Zea mays*, gamma radyasyonu, depolama, mitoz, mayoz, kromozom anormalliği

## Introduction

Chromosomal rearrangements are one of the most frequently produced classes of mutation that results from the action of both physical and chemical mutagens (1). Nowadays induced mutation has become an established tool in plant breeding to supplement existing germplasms and to improve cultivars in certain specific traits. Induced mutations are caused by exposing the biological material to various physical and chemical mutagens.

Gamma irradiation is one of the main physical mutagens for mutation studies in plants. Mutagens have been effective to decrease the mitotic index (2). Gamma irradiation can induce beneficial as well as deleterious impacts on the chromosomes of crop plants (3,4) and so there is a need to predict the most beneficial dose of gamma rays for improvement of specific traits of crop plants. In higher plants, chromosomal aberrations induced by irradiation have been utilized for many years in classical genetic studies (5) and more recently to provide starting material for gene isolation and mapping (6,7). Several researchers have reported chromosomal variations attained by gamma irradiation (1,8-12).

Generally, seeds are most commonly used for radiation. They offer a number of advantages. They are easy to handle and store, and can be maintained for an extended period of time in a vacuum, almost free of oxygen, as well as under high pressure of oxygen or other gases (13).

Seeds are stored under optimal storage conditions (low temperature and low seed moisture content) to prolong the seed viability. The deterioration of the stored seed is a natural phenomenon and the seeds tend to lose viability even under ideal storage conditions (14).

Cytogenetical investigation is one of the best-documented experimental proofs for the elucidation of the mode of speciation on different groups of plants (15). Cytogenetical changes occurring due to the effect of age of seed on gamma ray induced chromosomal anomalies have been described in detail by various researchers (16). Nilan and Gunthardt (17) reported that the germinability of seeds decreases and the frequency of chromosomal anomalies increases with the age and X-ray dose.

Generally, in the NEPZ (North-East Plain Zone) conditions of India, farmers prefer traditional farming. They cultivate the crops and after harvesting they consume these seeds and store the remaining seeds for planting in the next Kharif season. Storage of seeds affects not only the morphological traits but also the genetic structure of seeds.

Innovative research on genetic amelioration of kharif maize at the University of Allahabad has been carried out to assess the efficiency of gamma irradiation and ageing treatments and to elucidate the synergistic effects of combination treatments of gamma irradiation and ageing in somatic and gametic cells of inbred line of *Zea mays* L.

## Materials and methods

Seeds of the maize inbred CM-138 were obtained from the Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi and were irradiated at 4 doses of gamma rays: 200 Gy, 400 Gy, 600 Gy, and 800 Gy at the National Botanical Research Institute (NBRI), Lucknow, and after irradiation they were stored for 1 year under ambient conditions of Allahabad. Seeds were divided into 2 lots after gamma irradiation. Cytology of the first lot was studied immediately after gamma irradiation treatment and the second lot after individual treatment of storage (ageing) and combined treatment of gamma irradiation and storage (ageing).

For mitosis, seeds of inbred line of maize were germinated on wet filter paper in petri dishes. The treated seeds and control seeds (fresh seeds) were grown in petri dishes in the Plants Genetics Laboratory the day after storage treatments with 3 replications. The petri dishes were placed in an incubator maintained at  $25 \pm 1$  °C. For each treatment 10 seeds were used in each replication. The seeds from each sample were then germinated on moist filter paper in petri dishes. The excised root tips were first given pre-treatment in 0.1% colchicines solution for about 4 h at room temperature for maximizing the division of cells and fixed in a fresh solution (3:1) of absolute alcohol and acetic acid. After that, root tips were preserved in 70% alcohol and stored at 4 °C. The root tips were washed in distilled water and, after blotting with filter paper, they were stained in feulgen

(Leucobasic fuchsin) stain for 30 min for color development. Slides were prepared using chromosome squash technique with 2% acetocarmine.

For meiosis, seeds of maize inbred were sown in 3 replicates for each dose of treatment simultaneously. Suitable control seeds were maintained in distilled water and then they were sown under natural conditions to raise the  $M_1$  generation. At the time of flowering, young floral buds were fixed in 1:3 acetic acid: absolute alcohol solutions for 24 h after which they were transferred to 70% alcohol and stored at 4 °C. For each treatment 10 buds were used in each replication. For cytological analysis, slides were

prepared using chromosomal squash technique with 2% acetocarmine stain. Pollen grains were also stained with 2% acetocarmine to study pollen fertility. Photographs were taken from freshly prepared slides using a Nikon research photomicroscope.

## Results

### Mitosis

In *Zea mays*, the somatic complement consists of 20 chromosomes ( $2n = 20$ ) (Figures 1a and 1b). Seeds of the inbred line CM-138 were examined cytologically for mitotic abnormalities. Mitotic index was determined to be  $15.34 \pm 0.13\%$  and negligible

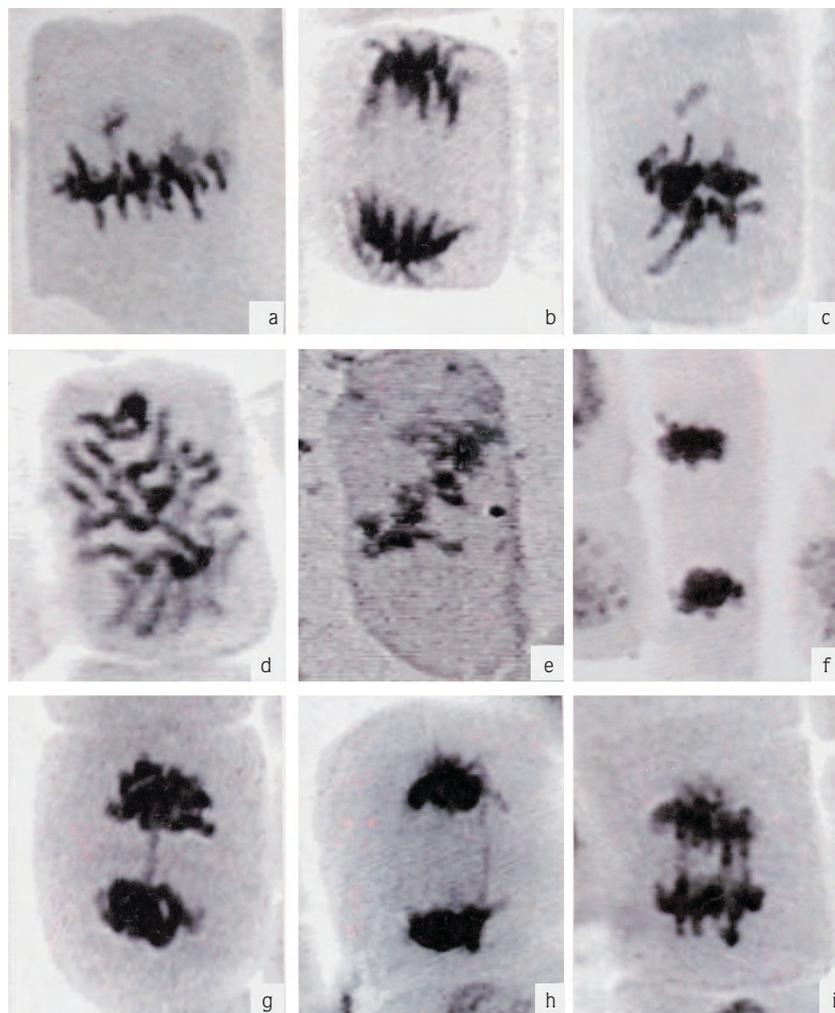


Figure 1. (Mitosis), (a) Normal metaphase, (b) Normal anaphase, (c) Clumping and precocious movement at metaphase, (d) Scattering at metaphase, (e) Disorientation at metaphase, (f) Stickiness at anaphase, (g) Single bridge at anaphase, (h) Double bridge at anaphase, (i) Multiple bridge at anaphase.

amounts (0.32%) of chromosomal aberrations were recorded in the control set. However, in the root tips of the gamma-ray exposed seeds, there was a gradual reduction in the mitotic index from  $15.34 \pm 0.13\%$  in control to  $14.92 \pm 0.33\%$  in 200 Gy,  $14.03 \pm 0.23\%$  in 400 Gy,  $11.83 \pm 0.57\%$  in 600 Gy, and  $7.11 \pm 0.73\%$  and 800 Gy, respectively, in experimental sets. On the other hand, in the 1-year ageing treatment set mitotic index was observed to be  $14.72 \pm 0.37\%$  while the combination treatments of gamma irradiation and ageing rendered a decreasing trend, which was recorded as  $15.17 \pm 0.08\%$  at lower dose (200 Gy+A<sub>1</sub>) to  $6.90 \pm 0.87\%$  at higher dose (800 Gy+A<sub>1</sub>). However, as far as the chromosomal aberrations are concerned, the total percentage of abnormalities in control and 1-year ageing treatment was recorded as 0.32% and 2.23%, respectively. Individual treatment of gamma irradiation displayed a synergistic impact on chromosomal aberrations and the percentage of abnormality displayed an increasing trend from 4.59%, 12.20%, and 20.78% up to 35.17% at 200 Gy, 400 Gy, 600 Gy, and 800 Gy doses. Among the observed abnormalities, bridge predominated with a rate of 4.14% at 800 Gy dose of gamma irradiation.

However, in combination treatments of gamma irradiation and ageing, the abnormality percentage was decreased as compared to individual treatment of gamma irradiation. It was registered as 4.04%, 10.44%, 17.72%, and 32.87% at 200 Gy+A<sub>1</sub>, 400 Gy+A<sub>1</sub>, 600 Gy+A<sub>1</sub>, and 800 Gy+A<sub>1</sub>, respectively. Furthermore, in the combination treatment sets, the maximum frequency of stickiness at 800 Gy+A<sub>1</sub> dose was registered as 6.29% at metaphase and 4.90% at anaphase (Table 1). Various types of chromosomal anomalies, such as clumping (Figure 1c), precocious movement (Figure 1c), scattering (Figure 1d), disorientation (Figure 1e), stickiness (Figure 1f), bridges (Figures 1g-1i), laggards, and fragmentation of chromosomes, were observed (Figure 1). In addition, an enhancement in dividing cells was also noted at respective doses in CM-138, except at the maximum dose of 800 Gy+A<sub>1</sub>, where the mitotic index in combination treatment was reduced as compared to the individual gamma-ray treatment sets. It is a clear cut indication of the mitoclastic and clastogenic property of gamma rays and ageing treatments, which is evident from the lowering of mitotic index and manifestation of spindle abnormality.

## Meiosis

Meiosis was perfectly normal in the control plants with 10 bivalents at diakinesis (Figure 2a) and at metaphase I (Figure 2b) and 10:10 separation at anaphase I (Figure 2c). However, the plants in both treatments, namely combined and individual treatments of gamma irradiation and ageing, displayed varying degrees of chromosomal abnormalities distributed in all phases of division. A dose-based increase in meiotic abnormalities was observed in both treatment sets. Although a number of abnormalities were present in both treatment sets, multivalents (Figure 2d), laggards (Figure 2e), stickiness (Figure 2f), forward movement (Figure 2g), univalents, cytoplasmic connections (Figure 2h), and bridges were more common in gamma irradiated sets, while stickiness, precocious movements, and laggards were more common in ageing and combined treatments of gamma irradiation and ageing. In addition, other abnormalities, such as unequal separation, scattering, and non-synchronous division, were also encountered (Figure 2). Table 2 gives a comparative account of various abnormalities observed under combined and individual treatment sets.

In the gamma-irradiated set, the percentage of abnormal chromosomes was increased along with increasing doses. The maximum frequency (37.04%) of chromosomal anomalies was observed at 800 Gy followed by 600 Gy (24.88%), 400 Gy (11.72%), and 200 Gy (4.67%) in inbred CM-138, respectively. Wide spectrum of chromosomal abnormalities, namely bridges, laggards, stickiness, clumping, forward movement, fragmentation, precocious movement, were encountered in the gamma treated sets. The percentage of chromosomal anomalies was lower at other treatment doses. Bridge formation was the most frequent abnormality in the gamma-ray treatment set at 800 Gy, followed by 600 and 400 Gy. Frequencies of lagging chromosomes or chromatids were observed at 800 and 600 Gy (Table 2). At high radiation doses, however, laggards were very frequent and this result could be a good indicator of chromosomal breakage.

In the ageing group, maximum chromosomal abnormality of 2.57% was recorded in one year of ageing (storage) treatment.

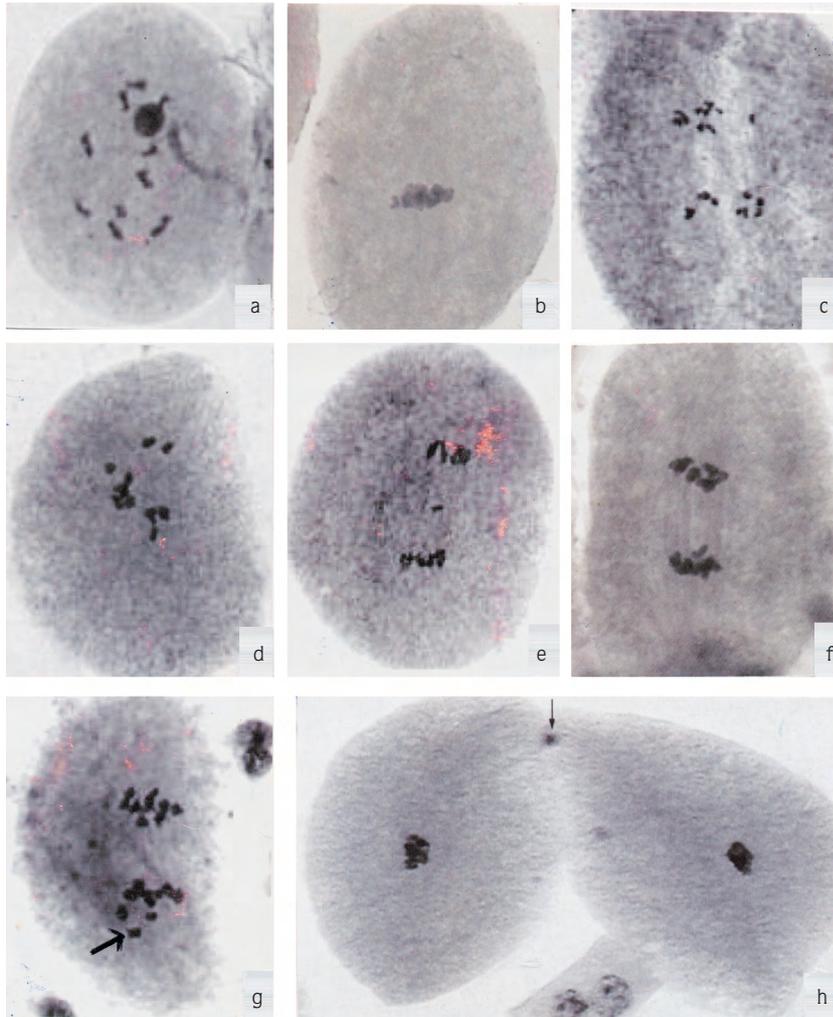


Figure 2. (Meiosis), (a) Normal diakinesis ( $n = 10$ ), (b) Normal metaphase I, (c) Normal anaphase I (10:10 separation), (d) Multivalent formation at metaphase I. {1 VIII: 1 VI: 2 II: 2 I}, (e) Laggards at anaphase I, (f) Stickiness at anaphase I, (g) Forward movement at anaphase I, (h) Cytoplasmic channel between 2 PMCs.

Gamma-ray and ageing treatments not only reduced the frequency of pollen mother cells but a wide spectrum of chromosomal anomalies has also been registered. However, a very interesting observation was recorded in the combination treatment; the total chromosomal abnormality percentage was reduced as compared to the individual gamma rays treatment at various doses (Table 2). In the combination treatment, maximum frequency of stickiness at 800 Gy+A<sub>1</sub> was registered as 6.29% at metaphase and 4.90% at anaphase while the maximum frequency of bridges (4.44%) was noted at 800 Gy. It was also observed that in the combination treatment set the frequency of bridges and scattering lowered considerably in comparison to the gamma-

ray treatment set. At 800 Gy dose, the frequency of bridges was observed as 4.44%, which decreased to 0.74% at 800 Gy+A<sub>1</sub> treatment dose. Similar results were also recorded at the other treatment doses.

The test for pollen fertility showed a very low percentage of sterile pollen grains in control sets. Pollen fertility was found to be significantly correlated with meiotic irregularities, as the meiotic abnormalities increased along with the dose of combined and individual treatment of gamma irradiation and ageing, the percentage of fertile pollen grains decreased. The gamma-ray treated samples revealed a greater decrease in pollen fertility as compared to the combination treatment.

Table 1. Mitotic indices and mitotic abnormalities in individual and combined treatment sets of ageing and gamma irradiation.

TI	Treatments	Metaphasic abnormalities (%)					Anaphasic abnormalities (%)					Total Cells Observed	T Ab (%) (Mean)	MI (%) (Mean $\pm$ S.E.)
		St	Pr	Un	Sc	Ot*	St	Lag	Br	Uneq	Ot**			
	Control	-	-	-	-	-	0.32	-	-	-	-	2034	0.32	15.34 $\pm$ 0.13
	After gamma irradiation (First Lot)													
	200 Gy	-	0.66	-	0.33	0.66	1.64	0.98	-	0.33	-	2044	4.59	14.92 $\pm$ 0.33
	400 Gy	1.05	1.39	1.05	1.05	1.74	1.05	0.70	1.39	0.70	2045	12.20	14.03 $\pm$ 0.23	
	600 Gy	2.35	1.96	1.57	2.35	2.75	1.57	1.18	2.35	1.57	2156	20.78	11.83 $\pm$ 0.57	
	800 Gy	3.45	3.45	2.76	4.14	4.83	2.76	2.07	4.14	2.76	2039	35.17	7.11 $\pm$ 0.73	
	After one year of individual and combined treatment of gamma rays and storage (ageing) (Second Lot)													
	A <sub>1</sub>	0.31	0.31	0.63	-	-	-	-	-	-	-	2126	2.23	14.72 $\pm$ 0.37
	200 Gy+A <sub>1</sub>	0.93	0.31	1.24	-	0.31	0.62	0.31	-	-	0.31	2122	4.04	15.17 $\pm$ 0.08
	400 Gy+A <sub>1</sub>	1.35	1.01	1.01	0.34	1.35	2.02	1.01	0.67	0.67	1.01	2098	10.44	14.16 $\pm$ 0.28
	600 Gy+A <sub>1</sub>	3.15	1.57	2.36	0.79	1.57	2.36	1.57	1.18	1.18	1.97	2167	17.72	11.72 $\pm$ 0.46
	800 Gy+A <sub>1</sub>	6.29	2.80	4.20	1.40	2.80	4.90	2.80	2.10	2.10	3.50	2071	32.87	6.90 $\pm$ 0.87

I. L.- Inbred Line, MI- Mitotic Index, T Ab- Total Abnormality, A<sub>1</sub> - One year aged seeds, 200 Gy+A<sub>1</sub> - 200 Gy treated with gamma rays+One year aged seeds, 400 Gy+A<sub>1</sub> - 400 Gy treated with gamma rays+One year aged seeds, 600 Gy+A<sub>1</sub> - 600 Gy treated with gamma rays+One year aged seeds, 800 Gy+A<sub>1</sub> - 800 Gy treated with gamma rays+One year aged seeds, St- Stickiness, Pr- Precocious movements, Un- Unorientation, Lag- Laggards, Br- Bridges, Sc- Scattering, Uneq- Unequal separation, Ot- other abnormalities, \*Clumping, Fragmentation, \*\*Micronuclei, Univalents, Multivalents, Secondary association.

Table 2. Meiotic abnormalities and pollen fertility in individual and combined treatment of ageing and gamma irradiation.

Treatments	Metaphasic (I/II) abnormalities (%)				Anaphasic (I/II) abnormalities (%)				Total PMCs Observed	T Ab (%) (Mean)	Pollen Fertility (%) (Mean $\pm$ S.E.)		
	St	Pr	Un	Sc	Ot*	St	Lag	Br				Uneq	Ot**
Control	-	0.31	-	-	-	0.31	-	-	-	-	2086	0.63	96.8 $\pm$ 0.11
After gamma irradiation (First Lot)													
200 Gy	0.33	-	0.33	0.33	1.00	1.33	1.00	-	-	0.33	2073	4.67	92.7 $\pm$ 0.43
400 Gy	1.56	1.17	0.78	1.95	1.56	1.56	0.78	1.17	0.39	0.78	2093	11.72	90.7 $\pm$ 0.25
600 Gy	2.49	2.49	1.99	2.99	3.48	1.99	1.49	2.99	1.99	2.99	2117	24.88	87.7 $\pm$ 0.33
800 Gy	4.44	3.70	4.44	5.19	3.70	2.96	1.48	4.44	2.96	3.70	2130	37.04	83.4 $\pm$ 0.28
After 1 year of individual and combined treatment of gamma rays and storage (ageing) (Second Lot)													
A <sub>1</sub>	0.64	-	0.32	-	0.32	-	-	-	-	-	2059	2.57	95.3 $\pm$ 0.17
200 Gy+ A <sub>1</sub>	0.94	-	-	-	0.63	1.57	0.63	-	-	-	2178	3.77	93.8 $\pm$ 0.37
400 Gy+ A <sub>1</sub>	1.92	1.15	1.53	0.77	1.15	1.92	0.77	-	0.38	0.77	2104	10.34	91.9 $\pm$ 0.17
600 Gy+ A <sub>1</sub>	2.97	2.48	0.99	1.98	1.49	2.97	2.48	1.49	1.98	1.98	2125	20.79	88.7 $\pm$ 0.17
800 Gy+ A <sub>1</sub>	5.88	5.15	4.41	2.94	2.21	4.41	2.94	0.74	2.21	1.47	2150	32.35	85.6 $\pm$ 0.51

I. L.- Inbred Line, PMCs- Pollen Mother Cells, T Ab- Total Abnormality, A<sub>1</sub> - One year aged seeds, 200 Gy+ A<sub>1</sub> - 200 Gy treated with gamma rays+One year aged seeds, 400 Gy+ A<sub>1</sub> - 400 Gy treated with gamma rays+One year aged seeds, 600 Gy+ A<sub>1</sub> - 600 Gy treated with gamma rays+One year aged seeds, 800 Gy+ A<sub>1</sub> - 800 Gy treated with gamma rays+One year aged seeds, St- Stickiness, Pr- Precocious movements, Un- Unorientation, Lag- Laggards, Br- Bridges, Sc- Scattering, Uneq- Unequal separation, Ot- other abnormalities, \*Clumping, Fragmentation, \*\*Forward movement, Univalents, Multivalent, Scattering, Cytomixis.

## Discussion

Induced mutation has benefited the plant breeders to explore genetic variations resulting from mutagens. Many reports are available for the successful use of mutation breeding in the production of new cultivars in many crops (18). During the present investigation, both combination and individual treatments elicited similar types of chromosomal anomalies, but the percentage of these anomalies and the total abnormalities induced differed between treatments. The induction of cytological disturbances in the mitotic as well as meiotic cells is of great value as it results in genetic damage that is passed on to the next generation.

Gamma irradiation test has been used widely for the monitoring of genetic effects. Even though the cell analysis was accomplished at various meiotic stages, the maximum alterations were observed at the dose of 600 and 800 Gy of gamma irradiation. The percentage of abnormal cells increased along with the increasing doses of gamma rays. Similar results have been reported by many researchers (4,8,9,16,19-21).

Disorientation and scattering of chromosomes may either be due to the inhibition of spindle formation or due to the destruction of spindle fibers formed. The behavior of these and of the laggard chromosome is characteristic in that they generally lead to micronucleus formation. Laggards and disturbed polarity might have appeared due to improper spindle functioning. At anaphase, about 50% of the cells displayed chromosomal bridges, often with fragments. These bridges may be the consequences of crossing over and associated with inversions or chromosome rings. In many studies, chromosome cluster, fragments, laggard, chromatin bridges, and micronuclei were observed as the effects of gamma irradiation (4,16,21). In some cases, the irregular outline of bridge formation through delayed separation of the chiasmata and also due to later replication of heterochromatin or chromatin stickiness was detected. Such stickiness might have been due to the radiation (3,22). Due to its easy identification, the use of bridges and fragments as indicators of the occurrence of chromosomal variations has been found to be an efficient method and favored in counting great numbers of cells. This criterion has been reported as being useful in

detecting abnormalities in seeds stored for long periods of time (23).

In modern agriculture, seed deterioration under storage is a problem (24). The ageing process is affected by genetic factors (25). Mitotic index values could decrease due to ageing in the root meristems of maize, which can also be attributed to mitotic inhibitions. Mitotic inhibition by ageing can be attributed to blocking of the mitotic cycle, which may result from a prolonged G<sub>2</sub> period or defective DNA synthesis. Reduction in mitotic index values indicates that ageing might have an adverse effect on the mitotic apparatus, thereby reducing the mitotic index. The result of the present study confirms the earlier reports (26,27).

The chromosomal aberrations in the root meristems and the pollen mother cells of the seeds also indicate that the ageing somehow alters the normal structure and function of chromosomes. Earlier researchers have reported increased chromosomal aberrations along with an increase in the storage periods of seeds from a wide range of species, such as peas (*Pisum sativum* L.) (28) and maize (*Zea mays* L.) (29,30), which is a fresh approach in defining the pattern of seed ageing in establishing the relationship between the rate of ageing and chromosome damage. Although chromosomal stickiness is one of the phenomena in chromosomal behavior that has been recorded for almost a century, adequate explanations are still lacking. The phenomenon was earlier identified by Koernicke (31) and characterized by intense chromosome clustering during any phase of the cell cycle. The term stickiness was firstly employed by Beadle (32) when he described the sticky aspect of chromosomes in a maize cell that had suffered a mutation. Chromosome stickiness has been documented to be due to genetic or environmental factors. Genetically controlled stickiness was also reported for wheat (33) and millet (34). Gaulden (35) postulated that sticky chromosomes might result from the defective functioning of 1 or 2 types of specific non-histone proteins involved in chromosome organization, which are needed for chromatid separation and segregation.

Several researchers reported that during ageing of seed under storage chromosomal aberrations, which mostly consist of bridges, laggards, fragments,

disturbed anaphase, stickiness, micronuclei-like chromatin fragments and point mutations, occur in various crops (30,36,37).

The combination treatment displayed the minimum frequency of chromosomal anomalies in comparison to individual treatment of gamma rays and ageing treatment. Chromosomal anomalies induced by gamma irradiation in seeds of maize inbred decreased while the ageing period of seeds increased. Similar results were obtained by Mishra and Raghuvanshi (16), and Nilan and Gunthardt (17).

As more and more abnormalities accumulate, the process of gamete formation is affected and it leads to non-viable gametes, which considerably reduce plant fertility. Studies on different plant species have shown that the decline in seed production is correlated with the meiotic irregularities (38).

Considering the results of these studies, genetic segregations should be carefully observed. Gamma rays and ageing treatments individually induced a number of chromosomal anomalies but, in combination, reduced the chromosomal anomalies considerably. From the present investigation, it is quite obvious that ageing inhibits the chromosomal damage induced by gamma radiation in the combination treatment. This study reports that ageing

(storage) of seeds after gamma irradiation must have led to genetic repairing in maize. Our results are in agreement with those reported by Mishra and Raghuvanshi (16), who worked on *Trigonella foenum-graecum*.

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