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RESEARCH PAPER

Optimization of gamma-ray irradiation dose for induced mutagenesis in field corn (*Zea mays* L.)

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Abstract: The present investigation was carried out to optimize the dose of gamma rays for practical mutation breeding application in field corn. An elite maize inbred line (PML-93) was irradiated with 10 different doses of gamma rays and seedling traits and growth parameters were evaluated by the paper towel and pot method. The observation of seed and seedling growth parameters such as germination percent, mean root length (MRL), mean shoot length (MSL), mean root dry weight (MRDW), mean shoot dry weight (MSDW), vigour index I (V-I), vigour index II (V-II) were recorded. The study revealed significant variations in all the traits under investigation in both the paper towel and the pot method. Out of 10 doses, the dose of 200 Gy was found optimum, it showed a 50 percent growth reduction (GR) in terms of most of the above growth parameters. Further, karyotype analysis showed that the chromosome breakages at one or two places as compared to the control. These aberrations may lead to heritable variation. Hence mutation breeding approach can be undertaken to create variability within this inbred line. The generated variability can be the best source to explore potential mutant line/s for the future breeding program.

Keywords: Gamma radiation · Inbred line · Karyotype · Optimum dose · Variability

Introduction

The mutation is a heritable change that alters the genetic makeup of an individual. It may occur naturally or can be induced. The mutation has been the single most important factor in evolution as the changes in genetic makeup produced are passed on to offspring and hence result in the appearance of new traits (Holme *et al*., 2019). The mutation, in some cases, may also result in reproductive isolation leading to speciation (Ma *et al*., 2021). Induced mutagenesis is being used for widespread application in the biological sciences, primarily for broadening the genetic base of germplasm in plant breeding, and more recently, as a tool for functional genomics (Mba *et al.*, 2010). Mutagens, the agents used to induce mutation, bring about changes in DNA sequences and consequently change the appearance, traits, and characteristics of the treated organism.

Mutagens are broadly classified as physical and chemical mutagens. Further, physical mutagens are classified as classical radiation mutagen, charged particle mutagen, and space radiation mutagen (Ma *et al.*, 2021). For seed propagated crops, the use of physical mutagens such as gamma rays were found to be the most appropriate strategy for achieving optimum genetic variation in the germplasm (Du *et al*., 2022). Earlier,

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gamma irradiation has been proved to be more effective and economical compared to other ionizing radiations because of their easy availability and power of penetration, as realized in corn (Al-Salhi *et al*., 2004), chickpea (Hameed *et al*., 2008), wheat (Sünnetcioglu *et al*., 1998), peas (Mashev *et al*., 1995), lentils (Chaudhuri *et al*., 2002), potato (Dale *et al*., 1997), citrus (Ling *et al*., 2008). However, the determination of the appropriate effective dose requires a detailed study of plant growth parameters and their interaction with the mutagens (Shrivastava *et al*., 2021).

Selection of the dose that could produce high mutation rates and may create desired mutant, is the critical and prerequisite step in the mutation breeding approach (Layek *et al*., 2021). The optimum dose might vary with the plant species, variety, *etc*. Hence, standardization of the mutagen dose is the first key step to getting a high mutation rate and assessing of radio sensitivity of the target genotype (Ahloowalia *et al.*, 2004). Sidhya and Pandit (2015) recorded dose-dependent retardation in biological parameters *viz*., seed germination, and plant survival, of snake gourd and reported 200 Gy was as the LD50 indicating a less damaging effect at lower doses on the genetic material. Considering the above facts, the induction of mutations to improve kernel size in the inbred line, PML 93, was contemplated. The PML 93 is a conventionally derived inbred line; has excellent general combining ability, and high yield *per se* (3.5 t/ha). It has all the required desirable characteristics to be used as a female parent in the hybrid breeding program (Mukri *et al*., 2021). However, the kernel size of PML 93 falls into the small category, as per the maize DUS descriptor (Das *et al.*, 2006), which is becoming a limitation in commercial hybrid seed production and limiting farmers' preference for it. Hence, an experiment was conducted to determine the appropriate dose of acute gamma irradiation producing 50% lethality or 50% growth reduction (considered as LD50 or GR50) in maize inbred line, PML 93 for inducing genetic variability and to correlate it with the growth parameters.

Materials and methods

Mutagen treatment

The inbred lines PML 93 (KDMH-176-5-1-1-B-B), a medium maturing inbred line, used in the active hybrid development program of ICAR-IARI was targeted for mutagenesis. The seeds of the test inbred line were obtained from the two different lots (Lot-I and Lot-II), grown during the post-rainy season, 2020 at two different locations (ICAR-Indian Agricultural Research Institute, New Delhi and ICAR-IARI, Regional Research Centre, Dharwad). These unirradiated seeds were subjected to a germination test by standard paper towel method and Lot II having 100% germination was sent to Bhabha Atomic Research Centre (BARC), Mumbai, India, for irradiating with gamma rays. A sample of 100 seeds each was irradiated with ten different doses of gamma rays *viz*., 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, and 700 Gy at BARC. A set of unirradiated seed samples were taken as a control for comparative analysis. These seed samples were pre-treated with Bavistin (2 g/kg) to prevent fungal infections during germination. The irradiated seed samples along with control were put to germination test using the paper towel method.

Experimental set-up

Paper towel method

A total of 20 seeds each for all 10 doses of gamma rays were grown in two replications under a growth chamber at 30°C for 10 days using a paper towel. One set of unirradiated seeds (PML 93) was taken as control. The seedling growth parameters were recorded 10 days after germination of the control seed. The growth parameters *viz*., percent germination [(number of germinated seeds/ total number of seeds) × 100], seedling length (root and shoot length) in cm, seedling fresh weight, and dry weight (oven-dry weight) in g, vigor index I [(mean root length + mean shoot length)] × percent seed germination) and vigor index II [(mean root dry weight + mean shoot dry weight)] × percent seed germination) were estimated using the data recorded on individual seedlings.

Pot method

The same set of experimental materials with three biological replications was sown in the pot (6-inch diameter) containing solarized soil medium. Here, a total of 30 seeds each for all ten (50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, and 700 Gy) dosages of gamma rays were placed in a completely randomized design containing ten seeds per replication, under natural conditions. Pots were irrigated as and when required with potable water. After 10 days, observations on growth parameters as that of the paper towel method were recorded.

Karyotype analysis

An enzyme-based method by Snowdon *et al*. (1997) was used to assess the chromosomal aberrations in the meristematic region of roots. Young growing root tips (1–2 cm) were fixed at the metaphase I stage in Carnoy's solution (cold ethanol: glacial acetic acid (3:1)). To capture the aberrations, first the fixative was rinsed (2 × 10 minutes in double distilled water) and roots were incubated in citrate solution (0.01M, pH 4.5) for 15 minutes. Root tips were treated with enzyme solution containing 5:1 cellulase (Onozuka R-10 cellulase, Yakult Honsha Co. Ltd., Japan) and pectolyase (Y23 pectolyase, Seishin Pharmaceutical Ltd., Japan) prepared in citrate solution (0.01 M, pH 4.5) for 1 hour 5 minutes at 37°C. Treated roots were scrambled on glass slides in Carnoy's solution and covered with a coverslip of the width of about 1 cm and visualized under the light microscope (LEICA DM 750, Germany) at 100 × (100 x/1.25 oil) attached with a high-resolution camera (LEICA DFC 3000 Germany).

The mean value of replications under each experimental setup was used for statistical analysis through SAS 9.3 v(SSCNARS, IASRI, New Delhi).

Results and discussion

Analysis of variance for growth parameters of gamma rays irradiated PML 93 genotype showed significant variation for all the studied parameters *viz*., root length, shoot length, root dry weight, shoot dry weight, vigor index-I, and vigor index-II, both for paper towel and pot method of growth analysis in a dose-dependent manner (Table 1 and 2). This indicated that the different doses used in the experiment have created a significant amount of variability due to cellular damage in PML 93.

Effects of gamma irradiation on germination percentage

The mean percent germination, root length, shoot length, root dry weight, shoot dry weight, vigor index-I, and vigor index-II showed a progressive reduction with the increase in dosage. Seedlings grown in a growth chamber on the paper towel method exhibited 100% germination for control as well as for all the gamma-ray doses except 400 Gy and 700 Gy, which recorded 80% germination (Table 3). The deviation in the germination percentage at 400 Gy and 700 Gy seems to be due to extraneous factors. On the other hand, the germination percentage of seedlings grown in the pot method was 90% in control and 73.3% in 50 Gy. At higher gamma-ray doses, there was a progressive reduction in germination percentage and it got reduced to 50% at 250 Gy. The lowest germination was recorded at 400 Gy (13.3%) and the seed samples irradiated with 500 Gy and above showed no germination implying that these doses are lethal for PML 93 (Table 4).

Table 1. Analysis of variance for growth parameters of PML 93 under different doses of gamma irradiation for paper towel method

Sources of variation	df	MSS					
		Root length	Shoot length	Root dry weight	Shoot dry weight	Vigour index I	Vigour index II
Replication	1	0.00	0.86	0.00	0.01	6136.58	81.91
Treatment	10	131.22**	55.87**	0.0072**	0.04**	3502154.69**	844.38**
Error	10	2.63	1.71	0.00	0.00	59406.20	24.23

**: Significant at 1% probability.

Table 2. Analysis of variance for growth parameters of PML 93 under different doses of gamma irradiation for pot method

Sources of variation	df	MSS					
		Root length	Shoot length	Root dry weight	Shoot dry weight	Vigour index I	Vigour index II
Replication	2	3.20	7.41	0.00	0.00	231.01	1.16
Treatment	10	166.73**	268.60**	0.0002**	0.002**	3873.08**	17.11**
Error	20	2.80	1.38	0.00	0.00	72.17	1.06

**: Significant at 1% probability.

Table 3. Effect of Gamma-ray irradiation on maize seedling growth parameters for paper towel method

S.No.	Treatment	Germination (%)	Mean root length (cm)	Mean shoot length(cm)	Mean root dry weight (g)	Mean shoot dry weight (g)	Vigour index I	Vigour index II
1	0 Gy	100.0	26.2	15.85	0.19	0.48	4201.3	67.4
2	50 Gy	100.0	18.7	14.51	0.16	0.38	3318.4	54.1
3	100 Gy	100.0	12.0	11.66	0.14	0.33	2369.4	46.2
4	150 Gy	100.0	11.4	11.62	0.12	0.32	2302.4	44.7
5	200 Gy	100.0	9.7	11.59	0.14	0.31	2131.0	44.6
6	250 Gy	100.0	7.9	11.62	0.12	0.30	1955.0	42.2
7	300 Gy	100.0	4.2	7.64	0.10	0.24	1181.5	33.1
8	400 Gy	80.0	4.6	7.24	0.08	0.16	944.0	19.0
9	500 Gy	100.0	1.5	2.24	0.03	0.08	370.1	10.9
10	600 Gy	100.0	0.14	1.07	0.02	0.07	120.5	8.9
11	700 Gy	80.0	0.04	1.13	0.02	0.04	125.5	4.5
	Mean	96.4	8.8	8.74	0.10	0.25	1729.0	34.1
	SD	8.1	8.1	5.29	0.06	0.14	1323.3	20.6

Table 4. Effect of Gamma-ray irradiation on maize seedling growth parameters for pot method

S.No.	Treatment	Germination (%)	Mean root length (cm)	Mean shoot length(cm)	Mean root dry weight (g)	Mean shoot dry weight (g)	Vigour index I	Vigour index II
1	0 Gy	90.0	16.5	20.9	0.02	0.05	105.9	6.74
2	50 Gy	73.0	15.3	20.3	0.02	0.05	78.2	5.25
3	100 Gy	64.3	15.4	19.5	0.01	0.04	42.4	3.52
4	150 Gy	63.0	12.8	17.2	0.01	0.04	42.7	3.43
5	200 Gy	56.7	12.5	16.9	0.01	0.03	25.6	2.41
6	250 Gy	40.0	2.6	6.4	0.00	0.03	16.6	1.36
7	300 Gy	36.70	0.13	0.73	0.00	0.00	2.8	0.15
8	400 Gy	13.3	0.1	0.67	0.00	0.00	0.0	0.07
9	500 Gy	0.0	0.0	0.0	0.00	0.00	0.0	0.00
10	600 Gy	0.0	0.0	0.0	0.00	0.00	0.0	0.00
11	700 Gy	0.0	0.0	0.0	0.00	0.00	0.0	0.00
	Mean	96.4	39.8	6.9	9.33	0.01	0.02	28.57
	SD	8.1	32.5	7.5	9.46	0.01	0.02	35.93

Effects of gamma irradiation on growth parameters

Among in the seedlings grown in the paper towel method, the mean root length (MRL) of the control was 26.2 cm. The increasing doses of gamma irradiation to seeds showed a progressive decrease in MRL. The MRL decreased from 18.7 cm at 50 Gy to 0.14 cm at 600 Gy and 0.04 cm for 700 Gy. In the pot-grown seedlings, the MRL was 16.52 cm in control, 15.33 Gy for 50 Gy, and the lowest (0.10 cm) at 400 Gy. A 50% reduction in MRL was recorded at 200 Gy. The mean shoot length (MSL) also showed reduction in mean values as a result of gamma irradiation as compared to the 15.85 cm MSL in control for the paper towel method and the reduced MSL were 14.51 cm, 11.66 cm, 11.59 cm, 11.62, 7.64 cm, 7.24 cm, 2.24 cm, 1.07 cm, and 1.03 cm for 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 500 Gy, 600 Gy, and 700 Gy dose of gamma rays, respectively. The dose of 300 Gy seems to reduce MSL by 50% in the paper towel method. In the pot method, there was not much reduction in MSL from control (20.89 cm) till the dose of 250 Gy 16.94 cm). However, at higher doses, there was a drastic reduction in MSL, which got reduced to 6.42 cm at 300 Gy to 0.77 cm at 400 Gy and to 0.67 cm at 500 Gy (Table 4).

It was observed that the mean root dry weight (MRDW) was 0.02 g for unirradiated seeds and decreased to 0.01 g which is 50% of the control at the dose of 150 Gy in pot germinated seedlings. Though the seeds irradiated with doses of 300 Gy and above showed germination, the MRDW values were negligible or close to zero. Likewise, in the paper towel experiment, a continuous decrease in MRDW values from 0.16 g to 0.02 g from50 Gy to 700 Gy compared to the control (0.19 g) was also observed (Figure 1). A similar trend of decreasing mean shoot dry weight (MSDW) with increasing gamma rays' dosage was noticed that ranged from 0.48 g (unirradiated) to 0.04 g (700 Gy) and 0.05 g (unirradiated) to 0.03 g (200 Gy) under paper towel and pot method of evaluation, respectively

The vigor index (VI) is a determinant of the interaction of multiple factors *i.e*., seed germination, shoot elongation, and their interaction with environmental conditions. Hence, the information derived by VI was taken to be more reliable than a single trait (Qun *et al.*, 2007). Vigor Index-I was also decreased to 3318.4, 2369.4, 2302.4, 2131.0, 1955.0, 944.0, 370.1, 120.5, and 125.5, respectively with increasing doses of irradiation from 50 Gy to 700 Gy as compared to control value (4201.3) in paper towel method. The same pattern was seen in pot-grown seedlings with values of 105.9 for control, 78.2 for 50 Gy, 42.4 for 100 Gy, 42.7 for 150 Gy, and 25.6 for 200 Gy, 16.6 for 250 Gy, and 2.8 for 300 Gy. The VI-I for doses 400 Gy, 500 Gy, 600 Gy, and 700 Gy were zero. A 50% reduction in the value of VI-I, was observed at the dose 100–150 Gy. Furthermore, VI-II values also decreased continuously as doses increased. The VI-II estimated were 6.74, 5.25, 3.52, 3.43, 2.41, 1.36, and 0.15 for doses of 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, and 400 Gy, respectively with control having a value of 6.74 for pot grown experiment. There was a complete cessation of seedling growth with zero vigor index- II value, beyond 400 Gy of gamma rays (Figure 2). However, the observed VI-II values for different doses of irradiation (50–700 Gy) were 54.1, 46.2, 44.7, 44.6, 42.2, 33.1, 19.0, 10.9, 8.9, and 4.5 with the control value of 67.4 for seedlings grown by paper towel method. In this case, a 50% reduction in VI-II was observed at the dose of 150 Gy.

GR50 doses slightly differed and ranged from 100 Gy to 300 Gy for different growth parameters studied here. To confirm the effect of these concentrations on the genetic component of the maize, karyotype analysis was conducted by taking meristematic root samples from

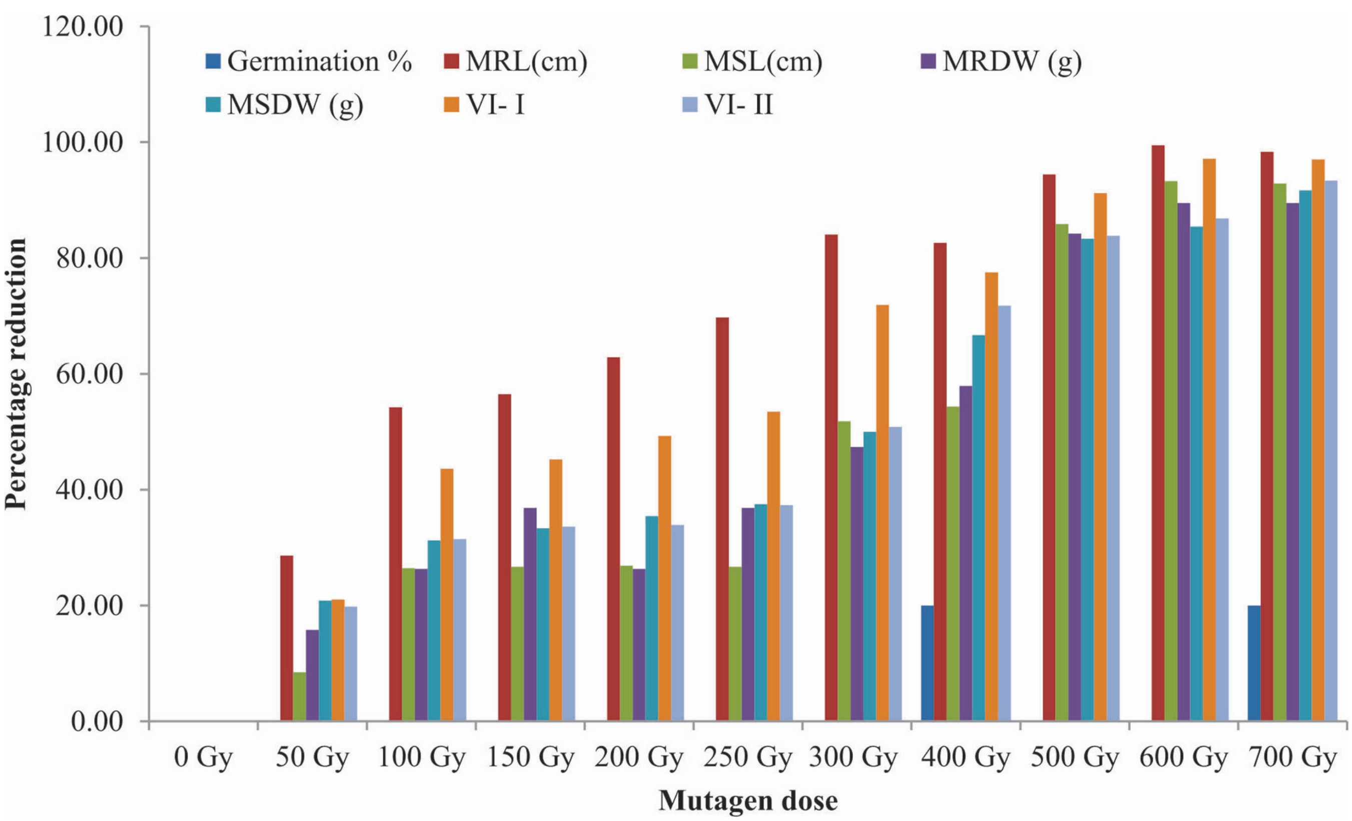

Figure 1. Percent reduction in growth parameters of PML 93, treated with Gamma rays under paper towel method of evaluation

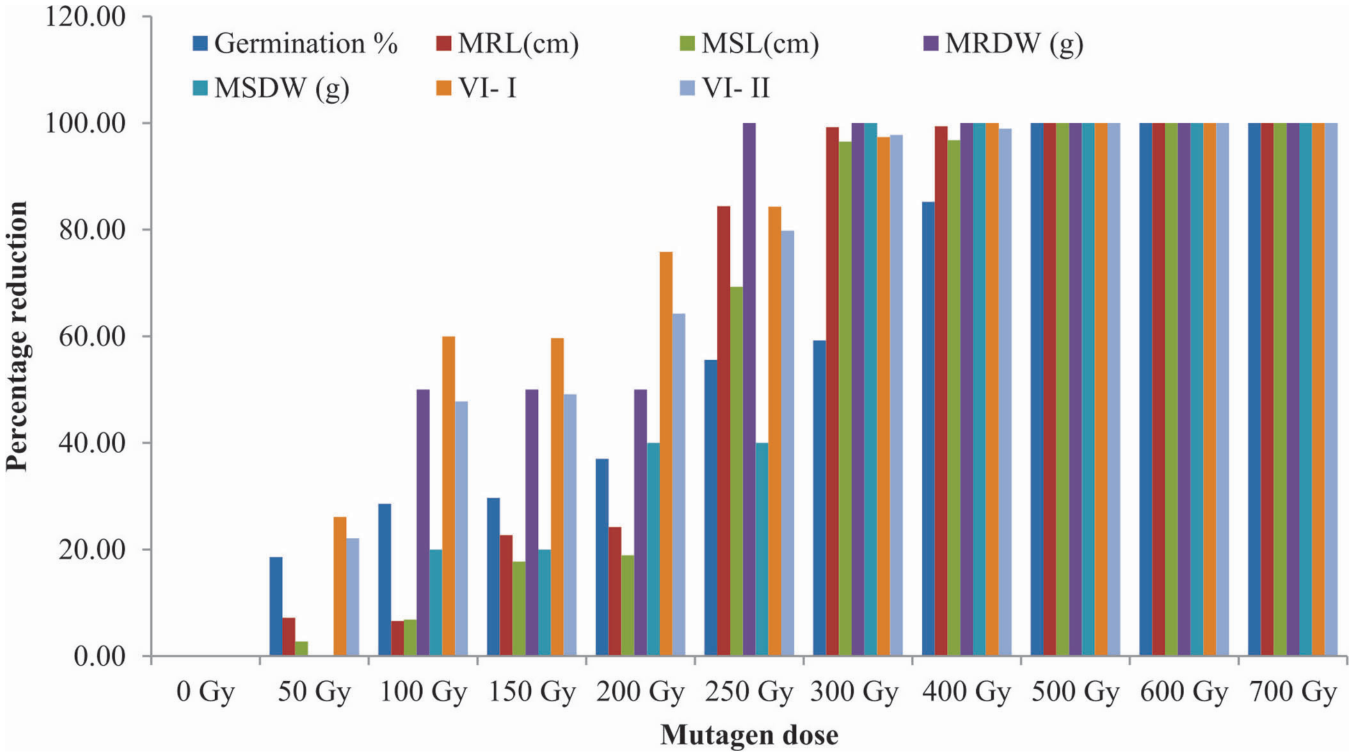

Figure 2. Percent reduction in growth parameters of PML 93, treated with Gamma rays under pot method of evaluation

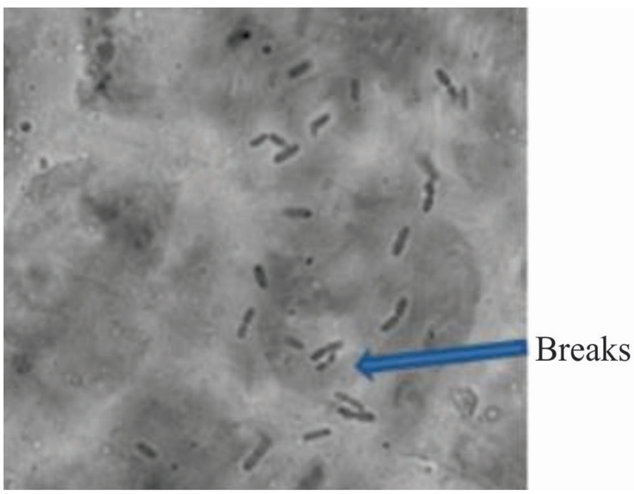

Figure 3. Chromosomal aberrations by the gamma rays at 200 Gy

different doses. It indicated that chromosomal aberration started with minor breakage in the chromosome with the dose of 200 Gy and onwards in gamma irradiation (Figure 3). As the dose increased, aberrations appeared more which justified lethality in maize. A drastic reduction in the establishment of healthy seedlings was observed when doses were increased from 200 Gy (Tables 3 and 4). By considering GR50 doses of all parameters and vigor indices, the 200 Gy can be considered as the actual GR50 dosage for the inbred line PML 93.

Conclusion

Optimization of gamma-ray doses for obtaining high mutation rates that may produce desired mutants is the basic requirement of a mutation breeding program. In the present study, the gamma-ray irradiation of field corn inbred induced significant variations in different growth parameters at 10 different doses ranging from 0 Gy to 700 Gy. Among the 10 doses of Gamma rays employed in the study, a dose of 200 Gy could produce a 50% growth reduction and hence it was decided to use 200 Gy as a GR50 dose to create genetic variability in the inbred line, PML 93. The irradiation of the seeds with gamma rays to generate mutants with desirable traits can be a potential source of novel genes for maize improvement programs.

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