RESEARCH PAPER

Quantitative Assessment of LD50 and Mutagenic Effectiveness of Gamma Irradiation and EMS in Red Bhendi (Abelmoschus esculentus (L.) Moench)

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ABSTRACT

The present study was conducted to evaluate the effect of gamma rays and Ethyl Methane Sulphonate (EMS) on germination, seedling growth, biomass accumulation, and chlorophyll content in Red Bhendi (Abelmoschus esculentus (L.) Moench). Healthy seeds were treated with varying doses of gamma rays (100-1000 Gy) and EMS concentrations (0.1-1.0%). Based on germination reduction and mortality data, the LD50 values were determined as 400 Gy for gamma rays and 0.5% for EMS using probit analysis. The results revealed a dose-dependent response of both mutagens on all studied parameters. Germination percentage decreased progressively with increasing doses of gamma rays and EMS, indicating the inhibitory effects of higher treatments. However, moderate doses of gamma irradiation and lower concentrations of EMS exhibited a stimulatory effect on seedling growth parameters such as shoot length, root length, seedling length, and vigour index. Biomass accumulation in terms of fresh and dry weight also followed a similar trend, with improved performance at moderate treatments and significant reduction at higher doses. Chlorophyll content was enhanced at moderate doses but declined at higher levels due to damage to chloroplast structure and reduced photosynthetic efficiency. Overall, the study demonstrated that both gamma rays and EMS are effective mutagens for inducing variability in Red Bhendi, with moderate doses being more suitable for improving growth and physiological traits. The identified LD50 levels can be effectively utilized in mutation breeding programmes for developing improved okra varieties with enhanced performance.

Keywords: Red Bhendi, Mutagenesis, Gamma irradiation, EMS, LD50, Probit analysis.

Key findings: The study identified the LD50 values as 400 Gy for gamma rays and 0.5% for EMS in Red Bhendi, with both mutagens showing a clear dose-dependent effect on germination, growth, biomass, and chlorophyll content. Moderate doses stimulated seedling growth, vigour, and physiological traits, while higher doses significantly inhibited these parameters due to physiological and genetic damage. The findings confirm the effectiveness of mutation breeding for generating variability and improving crop performance.

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INTRODUCTION

Red Bhendi (*Abelmoschus esculentus* (L.) Moench), commonly known as lady's finger or bhendi, is an important vegetable crop belonging to the family Malvaceae. It is widely cultivated in tropical and subtropical regions due to its adaptability, short duration, and significant economic importance. Globally, okra production reached about 11.5 million tonnes in 2023, with India contributing nearly 62% of the total output; within the country, the crop is cultivated on approximately 561.22 thousand hectares producing 7,611.71 thousand tonnes with an average productivity of 13.56 t ha^{-1}, while in Tamil Nadu it occupies about 19.87 thousand hectares with a

production of 216.98 thousand tonnes and a productivity of 10.92 t ha^{-1} (FAO, 2023; Ministry of Agriculture & Farmers Welfare, 2024-25). The crop is highly valued for its tender, mucilaginous pods, which are consumed in various culinary preparations and are known for their rich nutritional composition, including vitamins, minerals, dietary fiber, and bioactive compounds such as phenols and antioxidants (Mohite and Gurav, 2019). In addition to its dietary significance, okra also possesses medicinal and industrial applications, making it a multipurpose crop with considerable economic potential (Gulzar *et al*., 2024).

Despite its importance, the productivity and genetic improvement of okra remain constrained by several factors. One of the major limitations is the narrow genetic base of cultivated varieties, which restricts the scope for selection and improvement through conventional breeding approaches. Okra is predominantly a self-pollinated crop, and although some level of natural cross-pollination occurs, the overall genetic variability available for breeding is limited (Gupta *et al*., 2016). Furthermore, the crop is susceptible to various biotic stresses such as pests and diseases, including yellow vein mosaic virus, as well as abiotic stresses like drought and temperature fluctuations, which significantly affect yield and quality (Kalyane *et al*., 2024). These challenges highlight the need for innovative approaches to enhance genetic variability and develop improved varieties with better yield potential and stress tolerance.

The effectiveness of mutation breeding largely depends on the type and dose of mutagen used. Lower doses of mutagens are generally associated with stimulatory effects on plant growth and development, whereas higher doses may lead to detrimental effects such as reduced germination, impaired growth, and decreased survival (Khan *et al*., 2025). Therefore, determining the optimum dose of mutagen, commonly referred to as LD_{50} (lethal dose for 50% survival), is a critical step in mutation breeding programmes. The LD_{50} value helps in balancing the induction of mutations with minimal biological damage, thereby increasing the chances of obtaining desirable mutants (Gupta *et al*., 2016).

Several studies have demonstrated the potential of mutation breeding in improving agronomic and yield-related traits in okra. Induced mutations have been reported to influence various morphological and physiological parameters, including plant height, number of branches, fruit length, number of fruits per plant, and seed yield (Mohite and Gurav, 2019). In addition, mutation breeding has been shown to enhance stress tolerance and adaptability, which are essential for sustaining crop production under changing environmental conditions (Khan *et al*., 2025). The generation of chlorophyll mutants and other visible phenotypic variations further indicates the effectiveness of mutagenic treatments in creating genetic diversity.

Chemical mutagens such as EMS have also been widely used to induce variability in crop plants. EMS is considered a highly efficient mutagen due to its ability to induce a high frequency of point mutations with relatively low chromosomal damage. This makes it particularly useful for the development of improved varieties with specific desirable traits (Gulzar *et al*., 2024). Studies have shown that EMS treatments can lead to significant variation in plant growth, flowering, fruiting, and yield parameters, thereby providing a valuable source of genetic variation for the breeding programmers. Gamma rays, commonly sourced from cobalt-60, possess high penetration ability and induce mutations through ionization and the generation of free radicals, which alter the DNA structure and function (IAEA, 2018). In Red Bhendi, gamma irradiation has been successfully employed to induce variability in key traits such as germination, plant height, flowering time, pod length, yield, and quality parameters. The induced mutations may result in both beneficial and deleterious effects; therefore, optimization of radiation dose is crucial to achieve desirable outcomes without causing excessive damage (Ahloowalia *et al*., 2004). Lower and moderate doses are generally effective in producing viable and heritable mutations. Hazra *et al.* (2021) and Sasipriya and Gangaprasad (2021).

Mutation breeding represents a valuable tool for overcoming the limitations of conventional breeding in okra. The use of physical and chemical mutagens such as gamma rays and EMS has proven effective in generating genetic variability and improving economically important traits. Continued research in this field will contribute to the development of improved okra varieties with higher productivity, better quality, and enhanced stress tolerance, thereby supporting sustainable agriculture and food security.

MATERIALS AND METHODS

Quantitative assessment of ld_{50} and mutagenic effectiveness of gamma irradiation and ems in red bhendi (*abelmoschus esculentus* (l.) Moench)

The present study was conducted for quantitative assessment of LD_{50} and mutagenic effectiveness of gamma irradiation and EMS in red bhendi (*Abelmoschus esculentus* (L.) Moench) under Gamma irradiation and EMS. Healthy, uniform, and disease-free seeds of the Erode local Red Bhendi genotype were collected from Erode district, Tamil Nadu, India, and used as the experimental material.

The seed materials were subjected to treatments with both gamma rays and EMS. Gamma irradiation was performed by using a gamma chamber at Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, and the seeds were irradiated with ^{60}CO source. The different doses were 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy, 800 Gy, 900 Gy, 1000 Gy.

For EMS treatment, the experiment was carried out in the department of Vegetable Science laboratory, SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Baburayanpettai, Chengalpattu, Tamil Nadu, India. Healthy seeds were initially pre-soaked in distilled water for 12 hours. Pre-soaking enhances the permeability of seed cells and facilitates the uptake of mutagens by activating metabolic processes within the seed. After pre-soaking, the seeds were treated with different concentrations of EMS solution for six hours with intermittent shaking to ensure uniform exposure to the mutagen. The EMS concentrations used in the preliminary experiment were 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, and 1.0%. After the treatment, the seeds were thoroughly washed under running tap water to remove residual mutagen before sowing. For the laboratory germination test, the treated seeds were placed on absorbent germination sheets and maintained under laboratory conditions to record germination percentage, seedling growth parameters and biochemical characters of red bhendi. The data collected were then subjected to ANOVA of significance level 5% ($\alpha \leq 0.05$), following transformation of the data through the use of computer software known as “CPCS”, developed by Singh and Cheema in 1985, according to the method recommended by Gomez and Gomez in 1984. When statistical differences were found, the least significant difference (LSD) test was used at 5% level of significance.

RESULTS AND DISCUSION

Effect of Gamma Irradiation on Red Bhendi

Determination of LD_{50} of Gamma irradiation

The response of red bhendi to gamma irradiation showed a clear dose-dependent increase in mortality (Table 1). Mortality increased from 20% at 100 Gy to 77% at 1000 Gy, with corresponding probit values ranging from 4.16 to 5.74. The LD_{50} value was estimated at **400 Gy**, where 50% mortality was observed and the probit value was 5.00 .

This result confirms that gamma radiation induces cumulative physiological and genetic damage, leading to increased lethality at higher doses. Similar LD_{50} ranges (300–500 Gy) have been reported in okra and other vegetable crops, indicating that moderate doses are optimal for mutation breeding (Khan et al., 2009; Singh and Datta, 2010). The increase in mortality with dose is attributed to chromosomal aberrations, DNA strand breaks, and disruption of metabolic processes (Sharma, 2005).

The estimation of LD_{50} is an essential step in mutation breeding because it helps to determine the optimum mutagen dose capable of inducing genetic variability with minimum biological damage. Similar observations were reported by Gupta *et al*. (2016), who emphasized that LD_{50} estimation provides a reliable basis for selecting appropriate doses of physical and chemical mutagens in crop improvement programmes. Based on the germination % in both the treatment and control sets, the lethal dose (LD50) in each treatment was estimated (Laskar et al. 2018).

Effect of Gamma irradiation on germination

The germination percentage of Red bhendi seeds showed a progressive decline with increasing levels of mutagenic treatments. The control treatment (T_0) recorded the highest germination of 100%, indicating the normal viability of untreated seeds. Among the treated seeds, germination gradually decreased from 83.33% in T_1 to 16.67% in T_9 and T_{10}, demonstrating the inhibitory effect of higher mutagen doses on seed germination. Moderate reductions were observed in treatments T_2 (66.67%), T_3 (58.33%), and T_4 (58.33%), while further decreases were noted in T_5 (50.00%) and T_6 (41.67%). The lowest germination percentages were recorded in T_9 and T_{10} (16.67%), indicating severe physiological damage at higher mutagen concentrations. Similarly, Gamma irradiation treatments also influenced the germination percentage of red bhendi seeds, with germination declining progressively as radiation doses increased, indicating

the inhibitory effects of high radiation levels on seed viability. The variation in germination percentage under different Gamma irradiation treatments is presented in Figure 2. The reduction in germination at higher doses may be attributed to damage to cellular structures, enzyme inactivation, and disturbances in metabolic activities during seed germination. Similar reductions in germination percentage with increasing gamma irradiation doses were reported by Suneetha *et al*. (2018) and Kalyane *et al. (*2024) in okra., Baghery *et al.* (2016), Laskar *et al.* (2018) in tomato, Hasan *et al.* (2020) in chilli.

Effect of Gamma irradiation on seedling growth parameters

Gamma irradiation significantly influenced seedling growth traits of red bhendi, including shoot length, root length, biomass accumulation, seedling length, and seedling vigor index (Table 2). A clear dose-dependent response was observed, with stimulatory effects at lower doses and inhibitory effects at higher doses.

The control (T_0) recorded a shoot length of 7.78 cm, root length of 7.04 cm, and the highest seedling vigor index of 1482.00. Among the irradiated treatments, T_2 exhibited maximum shoot length (9.18 cm), which was 117.99% over control, and root length (7.88 cm), representing 111.93% over control. Similarly, treatment T_3 also showed improved performance with higher shoot length (8.34 cm) and increased shoot fresh weight (254.12 mg), suggesting that moderate radiation doses positively influence biomass accumulation. Such stimulation has been attributed to increased synthesis of growth-promoting hormones such as auxins and gibberellins under mild stress conditions (Kumar et al., 2013).

However, higher doses of gamma irradiation (T_8–T_{10}) resulted in a drastic reduction in all growth parameters. Shoot length decreased to 3.50 cm in T_8 and 3.30 cm in T_{10}, while root length declined sharply to 3.00 cm and 1.35 cm, respectively. The seedling vigor index dropped from 1482.00 in control to 66.40 in T_{10}, indicating severe growth inhibition. This reduction is primarily due to radiation-induced damage to cellular structures and physiological processes.

The inhibitory effects at higher doses may be attributed to several factors, including suppression of mitotic activity, chromosomal aberrations, DNA damage, and disruption of metabolic pathways (Kiong et al., 2008; Sharma, 2005). Additionally, reduced photosynthetic efficiency due to chlorophyll degradation further contributes to poor growth performance (Ashraf et al., 2003).

Biomass accumulation followed a similar trend, with moderate doses maintaining or slightly enhancing shoot and root weights, while higher doses caused significant reductions. This indicates that excessive radiation interferes with assimilate production and translocation within the plant system.

Seedling length, which integrates both shoot and root growth, was highest in T_2 (17.06 cm), confirming the beneficial effect of moderate irradiation. In contrast, higher doses led to a marked decline in seedling length, reflecting overall growth suppression.

The high coefficient of variation (CV) observed for root length (49.59%) and seedling vigor (75.39%) indicates substantial variability induced by gamma irradiation . Such variability is desirable in mutation breeding, as it provides a broader genetic base for selection of superior genotypes.

The reduction in seedling growth parameters at higher radiation doses may be attributed to chromosomal damage, disruption of cell division, and physiological disturbances caused by ionizing radiation. Similar findings were reported by Mohite and Gurav (2019), who observed that increasing doses of gamma radiation significantly affected morphological traits such as shoot length and root length in okra.

Likewise, Kalyane *et al*. (2024) reported that higher doses of gamma irradiation resulted in a significant reduction in germination and seedling growth due to mutagen-induced physiological damage.

Effect Gamma irradiation on chlorophyll content

The chlorophyll content of Red Bhendi seedlings was markedly influenced by the mutagenic treatments. In the control treatment (T_0), the chlorophyll a, chlorophyll b, and total chlorophyll contents were recorded as 0.36, 0.40, and 0.80 mg g^{-1}, respectively. Slight variations in chlorophyll content were observed in the lower treatments, with T_3 and T_4 maintaining relatively higher total chlorophyll values (0.80 mg g^{-1}), indicating a stimulatory effect at moderate doses. However, with increasing mutagenic treatments, a gradual decline in chlorophyll content was observed. The treatments T_5 and T_6 recorded moderate reductions in total chlorophyll content (0.50 and 0.40 mg g^{-1}, respectively), while further decreases were noticed in higher treatments where T_8, T_9, and T_{10} exhibited the lowest total chlorophyll contents (0.30,

0.20, and 0.20 mg g^{-1}, respectively). Similarly, gamma irradiation treatments also affected the biochemical characteristics of Red Bhendi seedlings, particularly chlorophyll content, with chlorophyll a, chlorophyll b, and total chlorophyll values declining progressively with increasing radiation doses. The variation in chlorophyll content under different gamma irradiation treatments is presented in Figure 3. The reduction in chlorophyll content at higher mutagen or radiation doses may be attributed to damage to chloroplast structures, inhibition of chlorophyll biosynthesis pathways, and reduced photosynthetic efficiency. Similar findings were reported by Mohite and Gurav (2019), who observed chlorophyll mutations and pigment variations in gamma ray-treated okra plants. Chlorophyll content of each plant of both the treatments and control was measured by using chlorophyll meter (SPAD-502) in µmol per m^2 of leaf surface. It was measured at full maturity stage, when leaves became dark green and fully expanded. Screening was done for chlorophyll and viable mutations. Chlorophyll mutations were categorized according to Rukesh *et al.* (2017). Generally, chlorophyll reduction is used as a confirmation criterion for successful mutation in plants and it indicates the probability of mutation occurrence in plant genome (Rajarajan *et al*., 2014).

Table 1. Determination of Lethal Dose (LD_{50}) of Gamma irradiation in Red Bhendi using Probit analysis

Dose	Log value for dose of mutagen	Total Number of plants	No. of plant dead	Mortality (%)	Probit
Control	0.00	30	0	0	0
100	2.00	30	6	20	4.16
200	2.30	30	9	30	4.48
300	2.47	30	12	40	4.75
400	2.60	30	15	50	5.00
500	2.69	30	16	53	5.08
600	2.77	30	17	57	5.18
700	2.84	30	19	63	5.33
800	2.90	30	20	67	5.44
900	2.95	30	22	73	5.61
1000	3.00	30	23	77	5.74

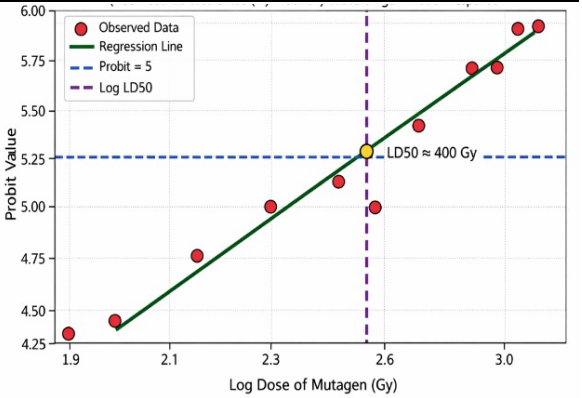


Figure 1. Probit analysis was performed to estimate the LD_{50} of gamma irradiation.

Quantitative assessment of ld_{50} and mutagenic effectiveness of gamma irradiation and ems in red bhendi (*abelmoschus esculentus* (l.) Moench)

Treatment	Shoot length (cm)	Shoot length (%) over control	Root length (cm)	Root length (%) over control	Shoot dry weight (mg)	Shoot fresh weight (mg)	Root Fresh (mg)	Root dry weight (mg)	Seedlings length (cm)	Seedling vigour	Seedling vigour (%) over control
T0	7.78	100.00	7.04	100.00	34.05	228.46	57.19	7.72	14.82	1482.00	100.00
T1	5.95	76.47	2.50	35.51	29.63	210.46	49.59	5.85	8.90	762.82	51.47
T2	9.18	117.99	7.88	111.93	25.68	177.96	41.62	4.71	17.06	1218.43	83.56
T3	8.34	107.19	6.62	94.03	35.90	254.12	62.51	7.70	14.96	854.81	57.67
T4	7.16	92.41	5.84	82.95	34.55	232.63	59.96	7.41	13.00	650.00	43.85
T5	6.22	79.94	7.00	99.43	32.13	217.46	53.64	7.32	13.22	472.09	31.85
T6	5.02	64.52	6.02	85.51	30.80	210.13	51.96	7.09	11.04	473.06	31.92
T7	6.00	77.12	5.20	73.86	28.95	201.13	45.29	6.37	11.20	239.90	16.18
T8	3.5	40.40	3.00	42.61	26.81	190.63	40.75	5.97	6.50	185.71	12.53
T9	4.00	51.41	1.85	26.27	24.75	170.46	41.24	5.39	4.85	69.26	4.67
T10	3.30	42.41	1.35	19.17	23.33	158.96	38.28	5.00	4.65	66.40	4.48
Mean	**5.86**	-	**4.76**	-	**29.25**	**202.39**	**48.48**	**6.28**	10.53	**499.24**	-
Sed	**1.98**	-	**2.34**	-	**4.17**	**29.03**	**8.44**	**1.06**	4.24	**376.39**	-
CV	**33.75**	-	**49.59**	-	**0.93**	**3.26**	**1.19**	**0.13**	40.26	**75.39**	-
CD	**0.18**	-	**0.14**	-	**14.288**	**14.34**	**17.42**	**16.96**	0.32	**17.42**	-

Table 2. Effect of Gamma irradiation treatments on seedling growth parameters of Red Bhendi

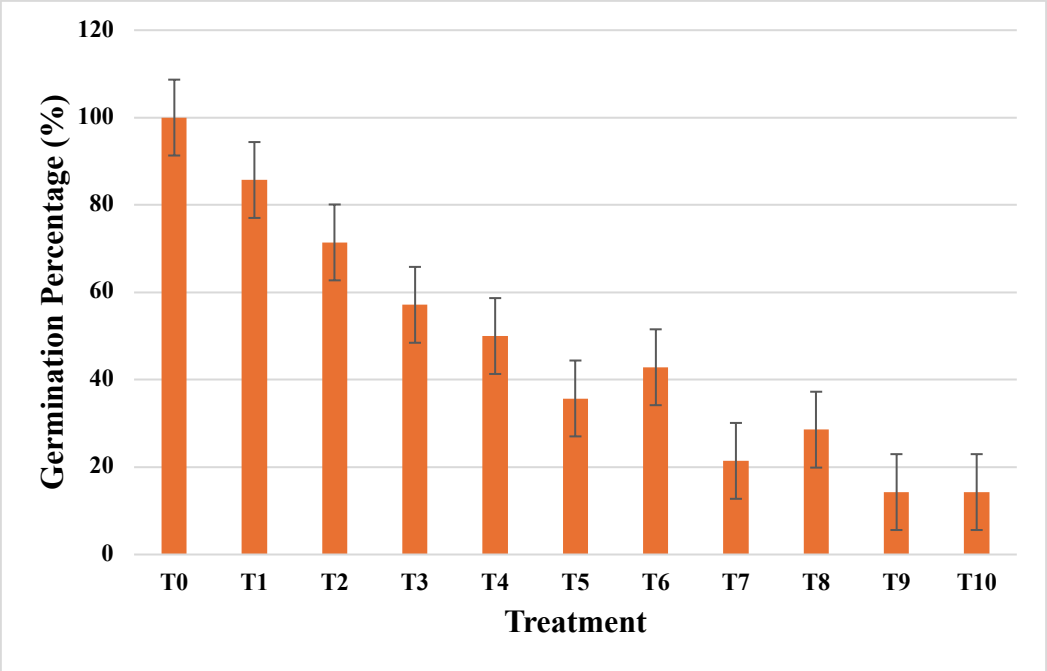


Figure 2. Effect of Gamma irradiation treatments on germination of Red Bhendi in the M_0 generation under laboratory condition

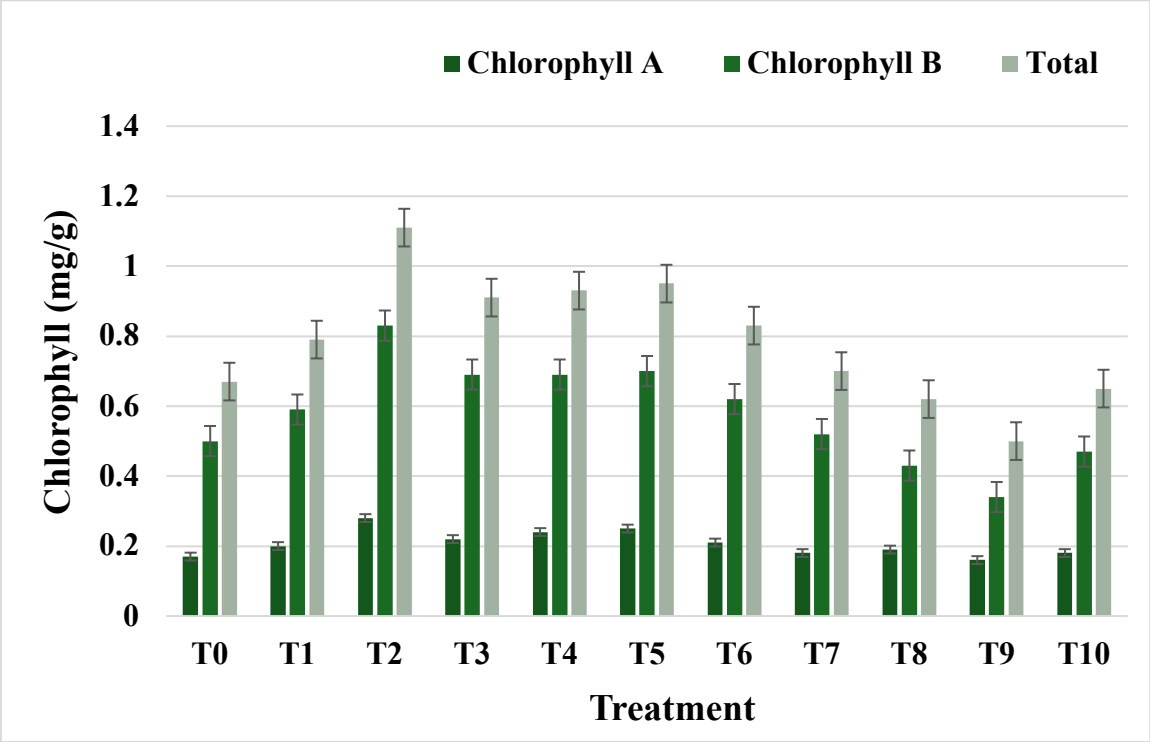


Figure 3. Effect of Gamma irradiation on biochemical parameters (Chlorophyll) of Red Bhendi at seedling stage

Quantitative assessment of ld$_{50}$ and mutagenic effectiveness of gamma irradiation and ems in red bhendi (*abelmoschus esculentus* (l.) Moench)

Effect of EMS mutagen treatments on Red Bhendi

Determination of LD$_{50}$ of EMS

The LD_{50} value for EMS mutagen treatment was estimated using probit analysis. Mortality percentage increased gradually with increasing concentrations of EMS. No mortality was observed in the control treatment, whereas the highest mortality (77%) was recorded at 1.0% EMS concentration. The LD_{50} value was estimated around 0.5% EMS concentration, where approximately 50% mortality occurred. The detailed mortality data are presented in Table 4, and the probit regression curve used to determine LD_{50} is shown in Figure 4.

EMS is one of the most commonly used chemical mutagens in mutation breeding because it induces point mutations by alkylating DNA bases. According to Gulzar *et al*. (2024), EMS is highly effective in inducing genetic variability and has been widely used in crop improvement programmes.

Effect of EMS on germination

EMS treatments significantly influenced the germination percentage of Red Bhendi seeds. The control treatment (T_0) recorded the highest germination percentage of 100%, indicating the normal viability and vigour of untreated seeds. However, germination percentage decreased gradually with increasing EMS concentration, demonstrating the detrimental effects of higher mutagen levels on seed viability. The treatments T_1 and T_2 recorded relatively higher germination percentages of 85.71% and 71.43%, respectively, indicating only mild effects of lower mutagen concentrations on seed germination. A further reduction in germination was observed in T_3 (57.14%) and T_4 (50.00%), suggesting moderate inhibitory effects of EMS treatments. More pronounced reductions were recorded in T_5 (35.71%) and T_6 (42.86), while T_7 (21.43%) and T_8 (28.57%) exhibited substantial decreases in germination percentage. The lowest germination values were observed in T_9 and T_{10} (14.29%), indicating severe inhibitory effects at higher EMS concentrations. The germination response under different EMS treatments is illustrated in Figure 5. Overall, the results clearly demonstrate a dose-dependent reduction in germination percentage, suggesting that increasing EMS concentration adversely affects seed viability and early seedling establishment due to physiological and cellular damage caused by mutagen exposure. Similar findings were reported by Kalyane *et al*., (2024), who observed a dose-dependent decrease in germination and survival percentage in okra under increasing EMS concentrations. For germination percentage, the result of present study coincides with the previous study in which the germination percentage was reduced with an increase in the concentrations of EMS as compared to control (Talebi *et al.,* 2012). The relationship between mutagenic dose and germination percentage was inversely proportional. Similar results were recorded by Singh *et al*. (1992), Sarada *et al*. (2015) and Prashant *et al*. (2020) in coriander.

Effect of EMS on seedling growth traits

Ethyl methane sulphonate (EMS) treatments significantly influenced seedling growth and biomass traits of red bhendi, exhibiting a clear dose-dependent response (Table 4). Considerable variation was observed among treatments for shoot length, root length, fresh and dry biomass, seedling length, and seedling vigor index.

The control (T_0) recorded the highest values for all parameters, with shoot length (7.96 cm), root length (7.66 cm), and seedling vigor index (1562.00). Among the EMS-treated populations, moderate concentrations such as T_1 and T_6 showed slight stimulation in growth, with shoot lengths of 8.18 cm (102.76% over control) and 8.20 cm (103.01% over control), respectively .

Similarly, root length and biomass parameters at moderate doses were relatively maintained or slightly enhanced compared to the control, indicating that mild EMS stress may promote growth through improved metabolic activity. Such responses have been reported in several crops, where low mutagen concentrations stimulate plant growth and development (Wani et al., 2011).

However, with increasing EMS concentration, a significant reduction in all growth parameters was observed. Higher treatments (T_8–T_{10}) showed drastic declines in shoot and root growth. Shoot length decreased to 3.50 cm (T_8) and 3.30 cm (T_{10}), while root length declined sharply to 3.00 cm and 1.35 cm, respectively. The seedling vigor index decreased from 1562.00 in the control to 77.47 in T_{10}, representing less than 5% of the control value .

Biomass accumulation also followed a similar trend. Shoot fresh weight and shoot dry weight were relatively higher at lower EMS concentrations but declined progressively with increasing concentration. Root fresh and dry weights were also significantly reduced at higher treatments, indicating impaired root

development and reduced nutrient uptake capacity. These reductions reflect the negative impact of high EMS doses on plant metabolic and physiological processes.

The inhibitory effects observed at higher EMS concentrations may be attributed to several molecular and physiological mechanisms. EMS acts as a potent alkylating agent that induces point mutations by modifying nucleotide bases, particularly guanine, leading to mispairing during DNA replication (Sega, 1984). This results in the accumulation of mutations that disrupt normal cellular functions. Additionally, EMS-induced damage interferes with protein synthesis and enzymatic activity, leading to metabolic imbalance and reduced growth (Talebi et al., 2012).

Furthermore, damage to meristematic tissues reduces mitotic activity, thereby limiting cell division and elongation. This ultimately leads to stunted growth and reduced biomass accumulation. Similar findings have been reported in mutation studies of various crops, where higher EMS concentrations significantly reduced seedling growth and vigor (Wani et al., 2011; Khan and Goyal, 2009).

Seedling length, which integrates both shoot and root growth, was highest at moderate treatments and declined sharply at higher concentrations, reflecting overall growth inhibition. The seedling vigor index followed the same trend, confirming that EMS has a strong influence on early seedling establishment.

The high coefficient of variation (CV) observed for root length (50.47%) and seedling vigor (74.28%) indicates substantial variability induced by EMS treatments. Such variability is highly desirable in mutation breeding programs, as it enhances the chances of identifying superior mutants with improved agronomic traits

The detailed data on seedling growth and biomass traits are presented in Table 5. The reduction in growth parameters at higher EMS concentrations may be due to mutagen-induced alterations in DNA structure, resulting in impaired cell division and metabolic disturbances. Similar observations were reported by Kumar *et al*. (2022), who noted significant reductions in seedling growth parameters in mutagen-treated plants. In general, ionizing radiation influenced growth and development of plants. Increased shoot length might be attributed to biochemical and physiological changes induced by gamma irradiation (Al-Bachir, 2014).

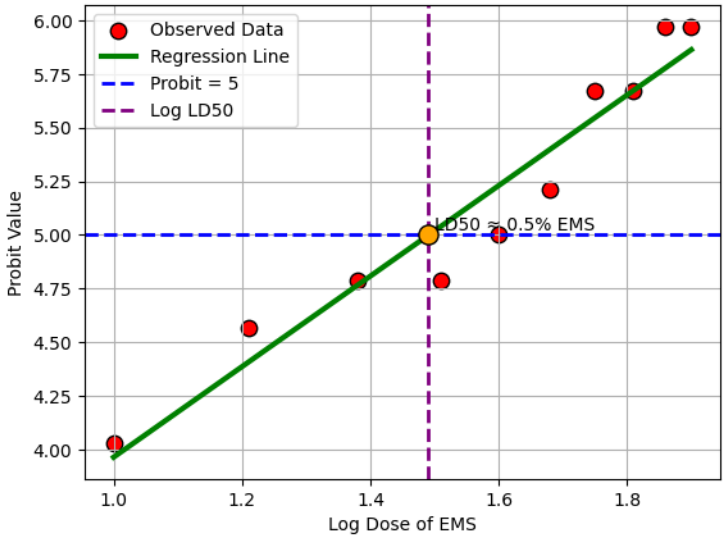
Effect of EMS on chlorophyll content

EMS mutagen treatments significantly influenced the chlorophyll content of Red Bhendi seedlings. In the control treatment (T_0), the chlorophyll a, chlorophyll b, and total chlorophyll contents were recorded as 0.17, 0.50, and 0.67 mg g^{-1}, respectively. An increase in chlorophyll content was observed in the lower EMS treatments, particularly in T_1 and T_2, where total chlorophyll values reached 0.79 and 1.11 mg g^{-1}, respectively. The highest total chlorophyll content was recorded in T_2 (1.11 mg g^{-1}), indicating a stimulatory effect of moderate EMS concentration on chlorophyll synthesis. However, with further increase in EMS concentration, a gradual decline in chlorophyll content was observed. Treatments T_3, T_4 and T_5 recorded moderate total chlorophyll contents of 0.91, 0.93, and 0.95 mg g^{-1}, respectively. A further reduction was observed in higher treatments, where T_6, T_7, and T_8 recorded total chlorophyll contents of 0.83, 0.70, and 0.62 mg g^{-1}, respectively. The lowest chlorophyll content was recorded in T_9 (0.50 mg g^{-1}), followed by T_{10} (0.65 mg g^{-1}), indicating inhibitory effects of higher EMS concentrations on photosynthetic pigments. The variation in chlorophyll content under different EMS treatments is presented in Figure 6. Overall, the results indicate that moderate EMS treatments enhanced chlorophyll content, whereas higher concentrations resulted in a decline, possibly due to disruption of chlorophyll biosynthesis and damage to chloroplast structures affecting photosynthetic efficiency. Mutagen-induced alterations in chlorophyll content are commonly observed due to disturbances in chloroplast development and pigment biosynthesis, and similar findings were reported by Suneetha *et al*., (2018) in mutation breeding studies in okra.

Table 4. Determination of Lethal Dose (LD_{50}) of EMS in Red Bhendi using probit analysis.

Quantitative assessment of ld_{50} and mutagenic effectiveness of gamma irradiation and ems in red bhendi (*abelmoschus esculentus* (l.) Moench)

Dose (%)	Log value for dose of mutagen	Total Number of plants	No. of plant dead	Mortality (%)	Probit
Control	0.00	30	0	0	0
0.1	1.00	30	7	23	4.26
0.2	1.21	30	9	30	4.48
0.3	1.38	30	11	37	4.67
0.4	1.51	30	13	43	4.82
0.5	1.60	30	15	50	5.00
0.6	1.68	30	17	57	5.18
0.7	1.75	30	19	63	5.33
0.8	1.81	30	20	67	5.44
0.9	1.86	30	21	70	5.52
0.10	1.90	30	23	77	5.74



ssion curve showing LD_{50} ment

Quantitative assessment of ld_{50} and mutagenic effectiveness of gamma irradiation and ems in red bhendi (*abelmoschus esculentus* (l.) Moench)

Treatment	Shoot length (cm)	Shoot length (%) over control	Root length (cm)	Root length (%) over control	Shoot Fresh (mg)	Shoot Dry (mg)	Root Fresh (mg)	Root Dry (mg)	Seedlings length (cm)	Seedling vigour	Seedling vigour (%) over control
T0	7.96	100.00	7.66	100.00	270.62	37.33	69.29	8.30	15.62	1562.00	100.00
T1	8.18	102.76	7.86	102.61	240.62	32.26	61.05	7.73	16.04	1336.61	85.57
T2	6.54	82.16	3.22	40.96	241.63	34.46	63.72	7.23	9.76	650.70	48.68
T3	7.04	88.44	7.92	103.39	230.62	32.16	53.55	6.41	14.96	872.62	55.86
T4	6.50	81.65	3.82	49.86	235.29	31.50	55.06	5.95	10.32	601.97	38.53
T5	6.74	84.67	5.62	73.36	224.62	27.46	42.03	4.92	12.36	618.00	39.56
T6	8.20	103.01	5.96	77.80	204.30	29.23	41.37	7.20	14.16	589.91	37.76
T7	6.00	75.37	5.20	67.88	195.29	23.40	39.40	4.12	11.20	280.00	17.92
T8	3.50	43.96	3.00	39.16	180.29	22.15	42.25	5.26	6.50	162.50	10.40
T9	4.00	50.25	1.85	24.15	166.96	26.16	37.29	4.37	5.85	97.46	6.23
T10	3.30	41.45	1.35	17.62	150.32	24.1	37.69	5.10	4.65	77.47	4.95
Mean	6.00	-	4.58	-	**206.99**	**28.28**	**47.34**	**5.829**	10.58	528.72	-
Sed	1.80	-	2.31	-	**32.78**	**4.27**	**10.00**	**1.27**	3.94	392.78	-
CV	30.07	-	50.47	-	**4.15**	**0.59**	**0.97**	**0.19**	37.31	74.28	-
CD	0.14	-	0.13	-	**15.83**	**15.10**	**21.13**	**21.80**	0.27	32.36	-

Table 5. Effect of EMS mutagen treatments on seedling growth and biomass traits of Red Bhendi in the M_0 generation.

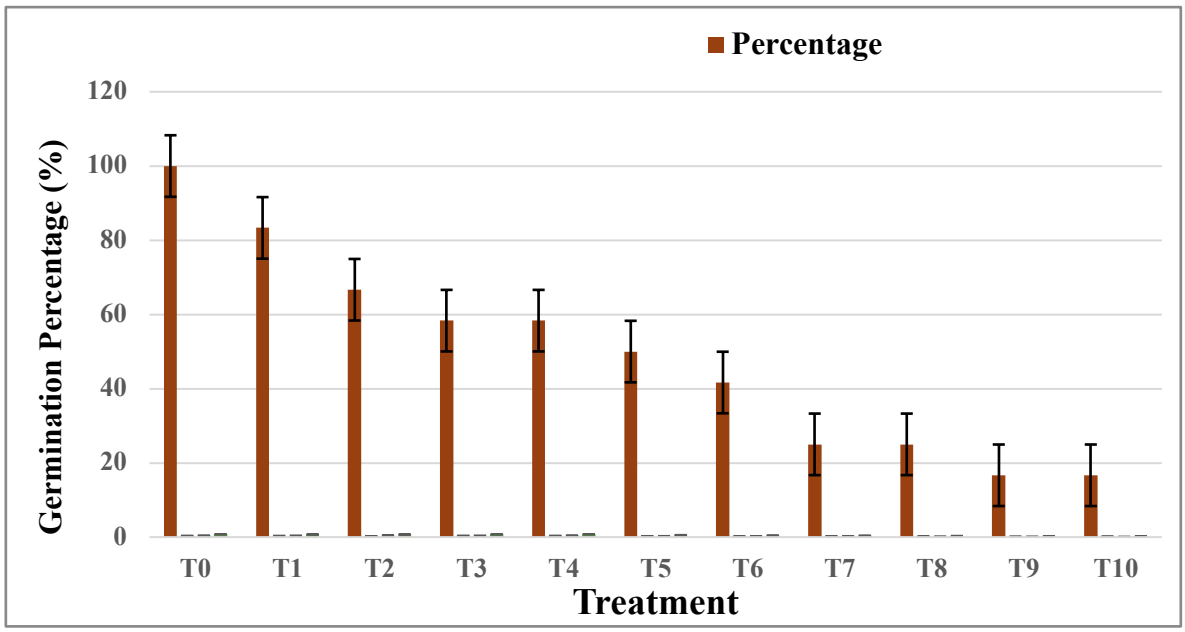


Figure 5. Effect of EMS mutagen treatments on germination of Red Bhendi in the M_0 generation under laboratory conditions.

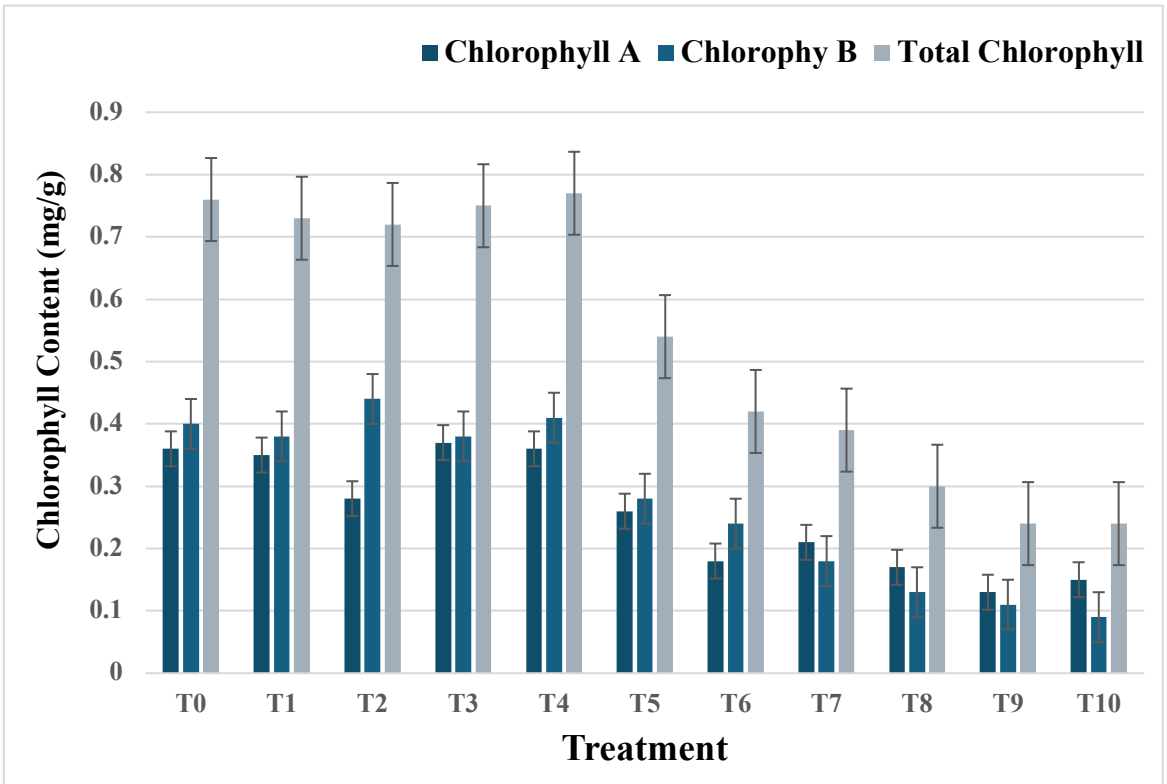


Figure 6. Effect of EMS on biochemical parameters (chlorophyll) of Red Bhendi at seedling stage

CONCLUSION

In this experiment, the effect of gamma radiation and Ethyl Methane Sulphonate (EMS) on generating variability in Red Bhendi is revealed. The LD_{50} values have been accurately assessed as 400 Gy in case of gamma rays and 0.5% for EMS. This

information serves as a baseline for optimizing the mutagenic doses to be used in breeding programs. The dose-dependent relationship was noted for all parameters tested; low to medium doses increased germination ability, vigour of seedlings, dry matter production, and the content of chlorophyll, while high doses had an adverse effect on the studied characteristics owing to negative impacts on cellular and physiological processes. Of particular interest are the data showing that the optimal dose range for enhancing beneficial responses in plants is 200-400 Gy for gamma rays and 0.2-0.5% for EMS. At the same time, the decrease in the studied parameters with increasing doses suggests high sensitivity of okra to mutagens and the necessity to optimize their concentrations. Overall, the results confirm the efficiency of induced mutations as an effective technique for widening the gene pool and developing new types of okra.

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