PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.6380

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RESEARCH ARTICLE

Unveiling the efficiency and effectiveness of two distinct mutagens in early mutant generations of sodic tolerant finger millet [*Eleusine coracana* (L.) Gaertn] genotype

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OPEN ACCESS

ARTICLE HISTORY
Received: 27 November 2024
Accepted: 11 January 2025
Available online
Version 1.0 : 09 March 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Monika S, Vanniarajan C, Vetriventhan M, Sakthivel K, Ramesh T, Meena S. Unveiling the efficiency and effectiveness of two distinct mutagens in early mutant generations of sodic tolerant finger millet [*Eleusine coracana* (L.) Gaertn] genotype. Plant Science Today (Early Access). https://doi.org/10.14719/pst.6380

Abstract

Finger millet is an essential small millet that has gained attention for its high calcium content and C_4 physiology. The self-pollination nature of the crop paves the way for the deliberate advent of candid variability, for which induced mutagenesis would be a suitable breeding method for the quick development of improved cultivars. In the present indagation, the sodic tolerant variety TRY1 finger millet was subjected to gamma rays and EMS to obtain early maturing genotypes. A preliminary experiment was conducted to investigate the LD_{50} value biological damages incurred by different mutagen doses, and mutagenic efficiency, effectiveness was estimated. Doses of gamma rays were used in the range of 100–500Gy. The EMS concentrations were 10 mM–50 mM. The LD_{50} values derived were 326.53 Gy of gamma rays and 15.36 mM EMS. Reduction in various quantitative traits became colinear with increased dose, irrespective of the mutagens. In the M_2 generation, the chlorophyll mutants such as *albino*, *xantha*, *chlorina*, *viridis*, *albomaculata* and *xantha viridis* were noticed. The highest noted chlorophyll mutant was *chlorina* (1.906 %–Gamma ray and 2.748 %–EMS), and *viridis* (0.701 %–gamma ray and 0.451 %–EMS) was with the least frequency. Mutagenic effectiveness was high in lower doses of the mutagen (1.65–250 Gy and 5.20–10 mM). The mutagenic efficiency was higher in lower doses of both the mutagens, concerning the mutagenic frequency and lethality (0.116-250 Gy, 0.085-10 mM) injury (0.336–250 Gy, 0.176–15 mM) and sterility (0.205–250 Gy, 0.206–10 mM). Thus, gamma ray and EMS at their minimum dose proved efficient in inducing variations.

Keywords

effectiveness; efficiency; EMS; gamma ray ; LD_{50}

Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn) is an essential small millet cultivated after sorghum, pearl millet and foxtail millet in Asian and African countries (1,2). Nutritionally, finger millet holds the highest calcium content (344 mg/ 100 g) compared to other cereals, along with a high amount of favourable amino acid spectrum, including methionine, cysteine and tryptophan (3). Being a C_4 crop and for its tolerance to drought and salt-affected soils, this millet plays a vital role in sustainable and climate-resilient agriculture. Finger millet is essential to provide food security and nourishment for millions worldwide, with an estimated yearly planting area of

4-4.5 million hectares and a total production of 5 million tonnes of grains. Out of the annual global estimate of finger millet production, India stands first by contributing 2.2 million tonnes of grain production (4). However, finger millet cultivation is majorly distributed in the country's arid and semi-arid regions, which have a high accumulation of salts, leading to salinity or sodicity of the soil. Salinity is a condition where the soil has high soluble salts in the plant's root zone (5). Sodicity is a condition where the soil has pH (>8.5), EC (<4.0 dsm^{-1}) and ESP (>15) due to high amounts of carbonates and bicarbonates. These unproductive soils adversely affect the soil properties that support plant growth and development. In India, 3.77 Mha is estimated to be sodic (6) and using sodic lands for cultivation is a demanding task to meet the upheaving population (7). Cultivation of identified sodic tolerant cultivars with inherent adaptation mechanisms would be more environmentally safe and suitable for these regions than undergoing large-scale amendments (8). Hence, developing finger millet varieties that are ideal for these regions with optimized maturity time, high yield, and intrinsic sodicity tolerance could allow farmers to expect improved productivity even in these challenging environments (4). Till date, TRY 1 finger millet released from Anbil Dharmalingam Agricultural College and Research Institute (ADAC & RI), Tamil Nadu Agricultural University (TNAU), Tiruchirappalli, Tamil Nadu, India is the popular finger millet variety suitable for sodic cultivation belt of India. The farmers of this sodic region highly prefer this variety, but it lasts 100–110 days. Since it was released in 1987 and is out of seed chain, it is essential to develop a new cultivar without compromising its sodic tolerance. Hence, the present study was attempted to develop and identify genotypes with early maturity without compromising yield and sodic tolerance. Varietal development of finger millet *via* recombination breeding, exerted through artificial crossing and hybridization, is complex because of its small floret size (9). Mutation breeding is an efficient breeding tool to develop desirable varieties within a short period compared to hybridization (10). It would be an alternative to hybridization for varietal development in finger millet (9,11). It can be used to eliminate an undesirable trait from a crop variety. Mutagens, including Gamma rays and EMS, produced desirable mutations in finger millet (12–15). Thus, this experiment aimed at creating variation in the TRY 1 finger millet variety using gamma rays and EMS, identifying the optimum dose/ concentration for obtaining desirable mutants, the dosage effect on the growth parameters and the efficiency and effectiveness of the mutagens in the M_1 and M_2 generation, obtained through induced mutagenesis.

Materials and Methods

Plant material

A finger millet variety TRY 1 in-house developed and released for cultivation in 1987 by Anbil Dharmalingam Agricultural College and Research Institute, TNAU, was used for the present study. TRY 1 finger millet variety is well adapted to sodic soil and yields 4010 kg/ha. However, the maturity duration is higher with 100–110 days.

Induction of mutation

The experiment employed two types of mutagens: gamma rays (physical mutagen) and Ethyl methyl sulphonate (chemical mutagen). These mutagens alter the genetic constitution of the material through their distinct course of action. Healthy, filled, dry TRY 1 seeds with 12 % moisture content were placed in butter paper covers and exposed to varying doses of gamma rays (100, 200, 300, 400, 500 Gy). Unirradiated seeds served as control (15). Gamma ray treatment was done using a dose irradiator at the Gamma chamber of the Indian Council for Agricultural Research-National Research Center for Banana, Tiruchirappalli. Low dose irradiator emits gamma rays from the source ^{137}Cs, with chamber temperature set at 26.8°C. The duration for all these treatments ranged from 8.36 to 41.50 mins with a dose rate of 11.959 Gy/ min. For chemical mutagenesis, five hundred seeds were pre-soaked for 8 hours to increase the physiological activation of seeds for absorption of the chemical mutagen. Those seeds were then subjected to different concentrations of EMS (10 mM, 20 mM, 30 mM, 40 mM, 50 mM) for four hours with intermittent shaking, after which they were decontaminated using sodium thiosulfate and washed in distilled water to remove EMS residues (16). Seeds soaked in distilled water were treated as control.

Fixation of optimum dose

The optimum dose or the concentration of the mutagen (LD_{50}), which produces desirable mutants while maintaining a desirable plant population, was determined using probit analysis (17). Probit analysis follows the function, which is the inverse of the Cumulative distribution function (CDF) or the quantile function, linked with standard normal distribution. The steps involved in determining the LD_{50} value using probit analysis are listed below:

- The dose or concentration of the mutagen is transformed to $\log_{10}$ values
- Mortality % for each treatment was rounded to the nearest whole number
- The corrected mortality % was calculated using Abbot's formula in Equation 1.

$$\text{Corrected mortality (\%)} = \frac{M_{observed} - M_{control}}{100 - M_{control}} \times 100 \quad \text{.....(Eqn. 1)}$$

- The corrected mortality values were rounded to the nearest whole number and transformed using the probit function.
- The probit values (Y-axis) were plotted against log10 values (X-axis)
- The straight line connecting the plotted points estimates the log10 concentration associated with a probit of five.
- Antilog of the log10 values from step 7 is used to find the LD_{50} value.

Experimental procedure

The mutated seeds were sown immediately in the field (M_1 generation) alongside control following Randomized Block Design (RBD) with three replications at the experimental farm, Anbil Dharmalingam Agricultural College and Research Institute, Trichy, Tamil Nadu, India, during the Summer of 2023. The field lies at a latitude of 10°45' N and a longitude of 78°6' E with an elevation of 85mSL. Standard agricultural practices were followed to ensure a healthy crop. Observations were made on germination percentage, root length, shoot length, plant height in vegetative and maturity stages, pollen and seed fertility. The results were assessed as a percentage reduction over control. Germination percentage, root length and shoot length were recorded in roll towels following ISTA rules (18). Pollen fertility was recorded from pollen grains collected from anthers of bloomed spikelets using 1 % potassium iodide.

In contrast, seed fertility was evaluated based on a percentage of filled and ill-filled grains as the effect of mutagens in the M_1 generation. Seeds from different treatments raised based on LD_{50} were collected from the M_1 generation as single plants and advanced to the M_2 generation as progeny rows. M_2 generation was raised during the Summer of 2024 at an experimental farm at ADAC &RI, Tiruchirappalli. In the M_2 generation, chlorophyll mutants were observed for about three weeks and characterized as suggested (19). Characteristics of different chlorophyll mutants obtained in M_2 generation are given in Table 1.

Table 1. Characteristics of different chlorophyll mutants obtained in M_2 generation

Chlorophyll mutants	Characterization
albino	White leaf-bearing seedlings survive up to 10-15 days and are lethal.
xantha	Seedlings with yellow-coloured leaves, not surviving beyond 10 days
chlorina	Pale green-coloured leaves in seedlings survive even after leaf drops.
striata	The leaf has green and yellow strips and is viable.
viridis	Viridine green becomes green later and survives.
xantha viridis	Seedlings become normal after the appearance of partly yellow and green colour leaf.
albomaculata	Green leaves bear white dots.

Mutation frequency is an indicator that estimates efficiency and effectiveness. The frequency of chlorophyll mutants on treatment with gamma rays and EMS, the effectiveness and efficiency of two mutagens, and the mutation rate were calculated using the formula in Equation 2-5 (20)

$$\text{Mutagenic effectiveness of gamma rays} = \frac{\text{Mutation frequency (Mf)}}{\text{Mutagen dose (Gy)}} \times 100 \quad \text{.....(Eqn. 2)}$$

$$\text{Mutagenic effectiveness of EMS} = \frac{\text{Mutation frequency (Mf)}}{\text{Mutagen concentration} \times \text{Time}} \times 100 \quad \text{.....(Eqn. 3)}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (Mf)}}{\text{Percent Injury (I)/ Lethality (L)/ Sterility (S)}} \times 100 \quad \text{.....(Eqn. 4)}$$

where, I:% of seedling injury in M_1/ M_2 generation, L:% of lethality in M_1/ M_2 generation and S:% of seed sterility in M_1/ M_2 generation

$$\text{Mutation rate} = \frac{\text{Sum of values of mutagen effectiveness or efficiency}}{\text{Number of treatments of particular mutagen}} \times 100 \quad \text{.....(Eqn. 5)}$$

Results

Optimization of LD_{50} values

The lethal dose at which the population's fifty percent survive is considered the optimum dose and obtained by performing probit analysis. In the present study, LD_{50} for the finger millet variety TRY 1 was obtained as 326.53 Gy for gamma rays and 15.74 mM for EMS (Table 2 & Fig. 1).

Table 2. Calculation of LD_{50} of gamma rays for the finger millet variety TRY 1

Treatments	Log_{10} of doses	Observed mortality percentage	Corrected mortality percentage	Empirical probit unit	LD_{50}
Gamma rays (Gray)					
Control	-	-	-	-	326.53
100	2.00	16.00	6.00	3.44	
200	2.30	29.33	20.90	4.19	
300	2.48	45.33	38.80	4.72	
400	2.60	60.00	55.20	5.13	
500	2.70	86.68	85.10	6.04	
EMS (mM)					
Control	-	-	-	-	15.36
10	1.00	36.00	31.90	4.53	
20	1.30	68.00	66.00	5.41	
30	1.48	74.00	72.30	5.59	
40	1.60	80.00	78.70	5.80	
50	1.70	94.00	93.60	6.52	

Effect of mutagens on biological parameters

The biological damages caused by the mutagen were studied using eight different growth parameters: germination percentage, root and shoot length, survival percentage, seedling height at 30 DAS and maturity, and pollen and spikelet fertility. The above data with transformed mean, percentage over control, and percentage reduction over control for better perception is presented (Supplementary Table 1 & Fig. 2). Germination percentage decreased with increasing doses of both mutagens. Seedling's ability to germinate reduced from 17.97 % (100 Gy) to 73.55 % (500 Gy) over control in the case of gamma rays and from 29.23 % (10 mM) to 81.30 % (50 mM) in chemical mutagen

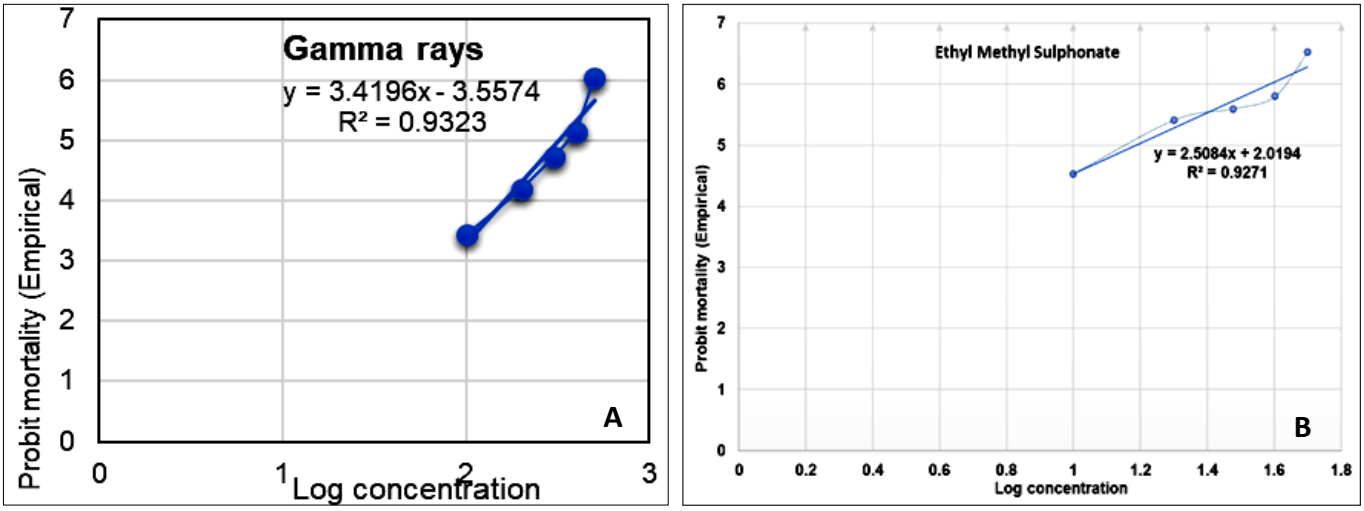

Fig. 1. Log doses vs probit for LD_{50} of **A**. Gamma ray treatment; **B**. EMS treatment.

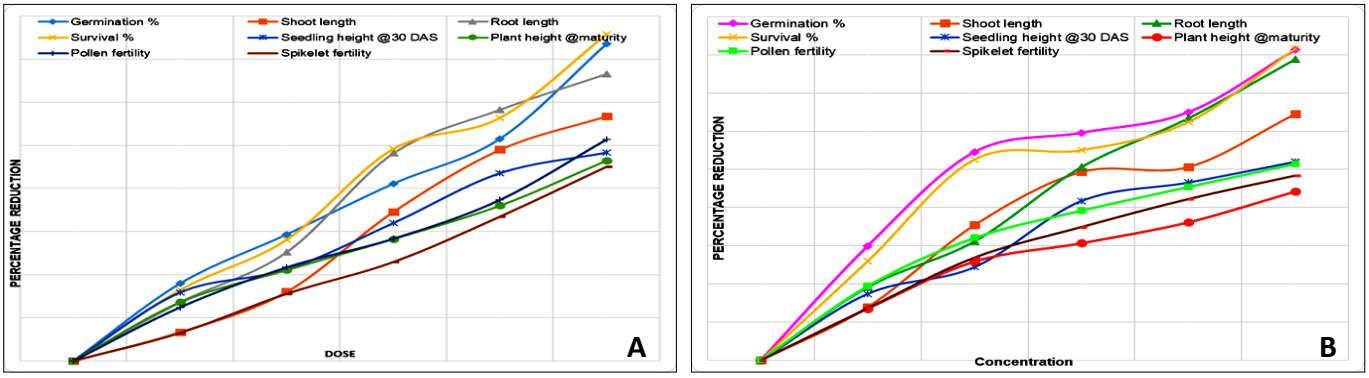

Fig. 2. Effect of mutagens on the biological parameters in M_1 generation as percentage reduction over control. **A**. gamma rays; **B**. EMS).

treatment. Seedling survival reduced linearly with an increase in the dose of both mutagens. At a higher dose of the gamma-ray treatment (500 Gy), the reduction percentage was 75.78 % and more seedlings survived at the lowest dose (100 Gy), where the reduction percentage was 16.46 %. When treated with different doses of EMS, the reduction percentage ranged from 25.96 % to 81.63 % at lower (10 mM) to higher doses (50 mM) treated, respectively. Reduction in shoot length ranged from 6.67 % (100 Gy) to 56.79 % (500 Gy) in gamma-ray treatment and with EMS, reduction in shoot length ranged from 13.92 % (10 mM) to 64.56 % (50 mM) in EMS treatment. Root length reduction was from 13.62 % (100 Gy) to 66.61 % (500 Gy) in gamma-ray and . Root length reduced from 19.15 % (10 mM) to 78.84 % (50 mM). At the vegetative stage, seedling height reduction ranged from 15.88 % in the lower dose (100 Gy) to 48.33 % at the highest dose (500 Gy) in gamma-ray treatment and 17.41 % to 51.99 % in EMS treatment of 10 mM and 50 mM respectively. At maturity, plant height reduced from a maximum of 46.50 % (500 Gy) to a minimum of 13.61 % (100Gy) in gamma-ray treatment and in EMS, the height reduced from a maximum of 44.21 % (50 mM) to 13.53 % (10 mM). Reduction in pollen fertility ranged from 12.42 % in the lowest gamma ray (100 Gy) treatment to 51.36 % at the higher dose (500 Gy) treatment. In chemical treatment with EMS, the reduction in pollen fertility ranged from 19.43 % (10 mM) to 51.47 % (50 mM). Spikelet fertility ranged from 6.52 % (100 Gy) to 45.09% (500 Gy) in gamma-ray treatment and in chemical treatment, the seed fertility ranged from 13.63 % (10 mM) to 48.36 % (50 mM).

Frequency and spectrum of Chlorophyll mutants

Observing chlorophyll mutations is the most convenient method to appraise mutagens' potency and genetic effect. The frequency of obtained mutants is noted for the preliminary selection of the intensity of the mutagen used and for calculating mutagenic effectiveness and efficiency. The chlorophyll mutants found in the experiment were *albino*, *xantha*, *chlorina*, *striata*, *xantha viridis* and *albomaculata* (Fig. 3). The frequency of chlorophyll mutants ranged from 2.55 % to 4.62 % in the TRY 1 genotype when both the mutagens were employed (Supplementary 2 and Fig. 4). The increasing order for frequency of chlorophyll mutants is *chlorina* > *xantha* > *striata* > *xantha viridis* > *albino* > *albomaculata* > *viridis*. In gamma ray treatment, the frequency of *albino* type was high at 250 Gy, *albomaculata* was high at 350 Gy and *chlorina* was high at 450 Gy. In EMS treatment, the type chlorina was high in all the concentrations. A lower frequency for the chlorophyll mutant type *viridis* was observed in all doses of both mutagens. There was a non-linear and linear relationship between the dose and frequency of chlorophyll mutants for physical and chemical mutagen, respectively.

Mutagenic effectiveness and efficiency

The effectiveness and efficiency of the mutagens are presented in Table 3 and Fig. 5. The efficacy of a mutagen relies on the mutation frequency produced per unit of the mutagen used. Comparing both the mutagens, EMS showed higher effectiveness than gamma rays. The mutagenic effectiveness of both mutagens ranged from 0.802 % to 6.365 %. The order of effectiveness in physical mutagen

Fig. 3. Classes of chlorophyll mutants obtained in M_2 generation.

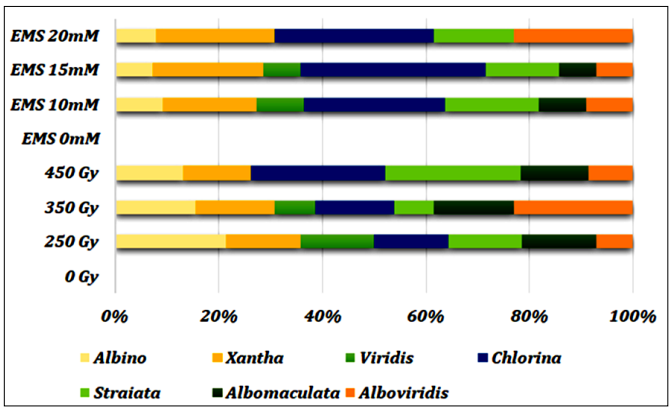

Fig. 4. Relative percent of chlorophyll mutants obtained in various treatments incurred.

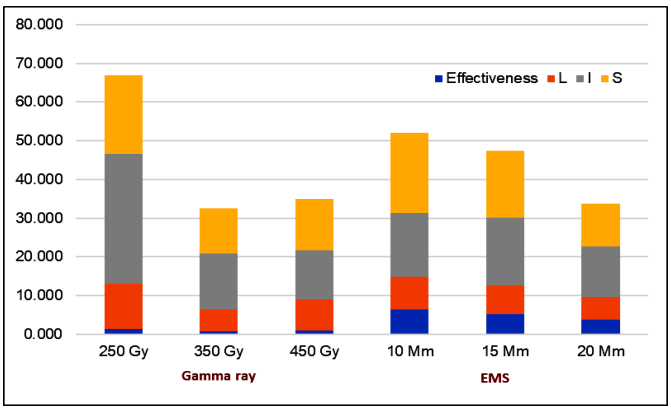

Fig. 5. Mutagenic effectiveness and efficiency of mutagens.

noted was 250 Gy> 450 Gy> 350 Gy. The rank for efficacy of the chemical mutagens is 10 mM> 15 mM> 20 mM. The efficiency of mutagen considers the mutants produced by each dose of the mutagen and the biological damage incurred by these mutants in the M_1 generation. The mutagenic efficiency considering lethality ranged from 5.70 % (350 Gy) to 11.60 % (250 Gy) in gamma-ray in EMS, the range was 5.70 % (20 mM) to 8.45 % (10 mM). Considering the injury, the highest efficiency noted was 33.60 % (250 Gy), and the lowest was 12.80 % (450 Gy) in gamma rays and in EMS, the range of efficiency was from 13.10 % (20 mM) to 17.60 % (10 mM). Considering the meiotic abnormalities, the range of efficiency was maximum from 20.50 % (250 Gy) to a minimum of 11.50 % (350 Gy) in gamma rays and the range of efficiency for EMS was from 20.60 % (10 mM) to 11.10 % (20 mM). Mutation rate indicates the frequency of mutations occurring in genes or nucleotide sequences. The mutation rate in terms of effectiveness was 0.011 in gamma rays and 0.05 in EMS. When calculated using efficiency, regarding lethality, the mutation rate was 0.08 in

Table 3. Mutagenic effectiveness and efficiency of the mutagens based on chlorophyll mutants in M_2 generation of finger millet

Treatments	% survival reduction on 30th day	% reduction height on 30th day	% reduction in Seed fertility	Mutation frequency (Mf)	Mutation effectiveness	Mutagenic efficiency		
	L	*I*	*S*			*L*	*I*	*S*
			Gamma rays (Gy)					
250	29.28	10.12	16.60	3.398	1.65	0.116	0.336	0.205
350	49.65	19.36	24.36	2.808	0.24	0.057	0.145	0.115
450	57.62	36.21	35.20	4.618	0.53	0.080	0.128	0.131
			EMS (mM)					
10	30.12	15.41	12.34	2.546	5.20	0.085	0.165	0.206
15	41.02	17.52	17.89	3.077	2.20	0.075	0.176	0.172
20	54.56	23.63	27.92	3.103	3.88	0.057	0.131	0.111

gamma rays and 0.07 in EMS. Concerning injury, the mutation rate was 0.203 in gamma rays and 0.157 in EMS. While calculating using sterility, the mutation rate was 0.150 in gamma rays and 0.163 in EMS (Fig. 6).

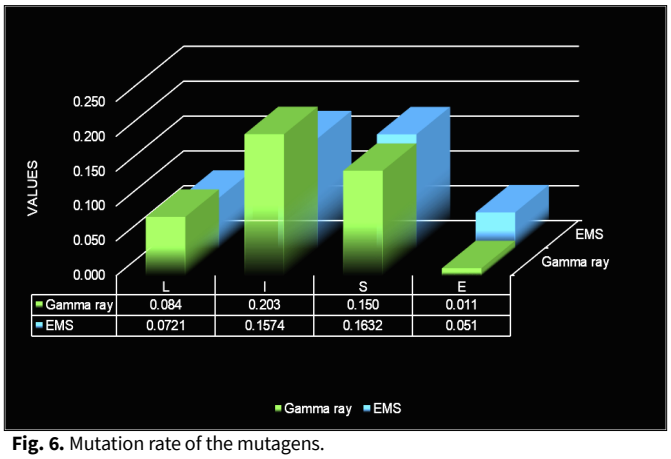

Fig. 6. Mutation rate of the mutagens.

Discussion

Optimization of LD_{50} values

The favourable outcome of mutation depends on the optimum dose of the mutagens used, the population studied, the efficiency of the mutagen and the mutation rate. Identifying the optimum dose of mutagen is required to minimize the loss of the mutagenized population and it depends on the biological damage incurred, treatment type and the environment (20). LD_{50} values arrived at 326.53 Gy in gamma rays and 15.36 mM for EMS. Similar results were obtained for EMS in barnyard millet and gamma rays in finger and kodo millet (16, 21, 22). This optimum dose can be used to mutagenize finger millet seeds of various varieties to create viable mutants and maintain the population for mutation breeding.

Effect of mutagens on biological parameters

Mutagens generally affect the growth and development of the seedlings, and the effect of mutagens varies depending on the type of mutagen and its different doses. Different growth and fertility-related parameters such as germination percentage, root and shoot length, seedling height at 30^{th} day and at maturity, and pollen and spikelet fertility were studied to estimate the biological damages incurred due to mutagens. A dose-dependent inverse linear correlation between mutagen dosage and these biological parameters was observed. Similar observations were reported in proso millet, barnyard, and finger millet (16, 20, 23).

The reduction in germination and survival was caused by the mutagen affecting the seed layer, causing cytological damage and thus disrupting metabolic activity (24). the decrease in germination may be due to the disruption in activity of the enzymes, α and β amylases, which break the starch granules and disperse energy for germination. However, it is to be noticed that the increasing doses cause chromosomal damage and interfere with cellular and physiological processes. The survival rate of the seedlings declines due to disturbed auxin production in the meristem and, most commonly, chromosomal abnormalities, which disturb the normal growth and development of the seedlings (26). The germination and survival rate reduction is higher in chemical treatment than in gamma rays since gamma radiation stimulates germination.

Comparing the reduction caused by both the mutagens, EMS showed the maximum reduction in the organ development of the germinated sprouts. These reductions occur due to limitations in protein synthesis within the embryonic cells, which hinders the transition of cells from the G_1 phase, ultimately slowing down the cell division in the root and shoot (24, 27). The alterations in the cytology of the root cells, such as the formation of anaphase bridges, laggards, and stickiness due to increased doses of the mutagen, may also be a reason for the reduction in sprout length (28). Mutagens disrupt the cytokinin response factors in *Arabidopsis thaliana*, producing smaller root meristem, fewer primary roots and etiolated seedlings (29).

The suppressive effects of the mutagen on plant height at the 30^{th} day and physiological maturity were investigated. These are often used as an index to resolve the impact of the mutagens in the first mutant generation. The reduction in plant height could be linked to the inhibitory effects of enzymes during the early growth stages, the production of growth inhibitors (30), changes in enzyme specificity, delays in the onset of the first meiosis (31), inhibition of cell division and elongation, and a breakdown in auxins like IAA (32). High doses of gamma-ray exposure in seeds interfere with protein synthesis, hormone regulation, leaf gas and water exchange, which could cause detrimental effects on plant height (33). Moreover, increased radiation doses affect carbon partitioning due to damage to radiosensitive cells responsible for carbohydrate transport in the phloem (34).

Pollen and spikelet fertility decreased with an increased dose of the mutagen. Pollen fertility is affected due to cell death in tapetal cells and defects in exine formation and ubisch bodies, as reported in rice. This is the result of a deficiency in *GA* and *gamyb* loci governing pollen fertility (35). Disturbance in the genetic equilibrium and physiology, chromosomal aberrations and decreased mitotic index also affect pollen fertility , affecting spikelet fertility(36). Disruption in hormones like auxin, jasmonic acid and gibberellic acid also affect spikelet fertility. With increasing mutagenic doses or concentrations, the biological damages increase, as evident from the eight traits observed in the M_1 generation.

A higher dose of the mutagen increases the genomic and chromosome abnormalities, resulting in biological damage. Higher doses of the mutagen disturb the mitotic task in the actively dividing meristem tissues and the seeds' moisture content, which also account for the reduction in these above biological parameters (34). However, observing higher abnormalities does not represent the appearance of desirable mutants. Hence, the efficiency and effectiveness of the mutagen in producing desirable traits are to be estimated using the chlorophyll mutants. These mutants do not provide a beneficial role in breeding and selection but play an important role in estimating the effectiveness and efficiency of the mutagens. Variations in

the sensitivity of the same crop may differ based on the genotype, the mutagen and the doses used.

Frequency and spectrum of chlorophyll mutants

Chlorophyll mutants appear at the early stage of the plant life cycle and later, some types develop with impaired growth and development. The frequency and range of chlorophyll mutants are noticed to estimate the efficiency and effectiveness of the mutagens (37). The different chlorophyll mutants in the study include *albino*, *xantha*, *chlorina*, *striata*, *viridis*, *albomaculata* and *xantha viridis*. The appearance of more than one type of chlorophyll mutants indicates the occurrence of simultaneous mutation in more than one locus (38). The presence of chlorophyll mutants was due to the disturbance in several genes governing the trait at distinct loci located in the proximal areas of the centromere (39, 40). The changes in chlorophyll biosynthesis, subsequent chlorophyll breakdown, and bleaching caused by lack of carotenoids and their preferential impact on genes involved in chlorophyll production are intimately linked to all of the reported chlorophyll mutants (41). In the present study, no linear correlation for the appearance of chlorophyll mutants was observed with an increase in mutagen doses in gamma ray treatment. Similar results were noticed by the various researchers (42, 43). In EMS mutagenesis, chlorophyll mutants are reported to increase with increased mutagen doses (44, 45). Chlorina type was found to be higher in both mutagenic treatments (38, 43). The lower frequency was found for the kind viridis in both the mutagen treatments. Thus, comparing the two mutagens, a higher frequency of chlorophyll mutants appeared in the gamma-ray treatment than in the EMS treatment. This may result from different mutagens and their dosages applied, genotypes and factors like duration.

Mutagenic effectiveness and efficiency

Estimating mutagenic effectiveness and efficiency exhibits the practical use of mutagens in various breeding programmes. Effectiveness denotes the number of mutations that occurred for the mutagen dose given and the sensitivity of the genotype. The efficiency of the mutagen gives the biological damages caused by the mutagen treatment and differs based on the parameters like lethality, injury and sterility that appeared in the M_1 generation. The efficiency and efficacy of mutagenic processes rely upon various factors such as genotype, cell cycle stage, physiological state of the propagule and the type of mutagen used. In the present study, mutagenic frequency and the mutagen dose remained unconnected, and a non-linear trend was observed in the gamma-ray treatment (40). Mutagenic effectiveness was high in lower doses of both the mutagen (46-48) and the reduction was non-linear in gamma-ray and was progressive concerning higher doses in EMS. Mutagenic efficiency concerning survival and plant height reduction was noted to have high values among all the mutagen treatments. Considering the seed fertility, efficiency was high in the lower doses of physical and chemical mutagen treatments. Higher efficiency at lower and moderate doses of mutagenic doses is due to relatively low biological damages caused than the higher dose (49). Similar results were found in barnyard, Kodo, and proso millet (43, 45, 51, 52). Comparing the two mutagens, gamma rays' efficiency was higher than EMS. This may be due to factors such as solubility, toxicity and chemical reactivity, which affect the efficiency of chemical mutagens (53). This suggests that even minor mutations brought about through lower mutagen doses have resulted in incredible outcomes in mutation breeding. A mutagen with a high effectiveness may not always exhibit greater efficiency, and vice versa (53). Mutation rate concerning effectiveness: EMS showed higher values than gamma rays, and in connection to efficiency (L and I), gamma rays showed higher mutation rates than EMS.

Conclusion

The present study was undertaken to develop variations in the TRY 1 sodic tolerant finger millet variety. Gamma rays and EMS were employed in the TRY 1 finger millet variety to optimize the mutagenic dose and compare their effectiveness and efficiency. The LD_{50} values for the mutagen gamma ray was 326.53 Gy and for EMS, 15.36 mM. The biological damages uncovered the effectiveness and efficiency of gamma rays and EMS, suggesting their wide application in finger millet mutation breeding. Both the mutagens proved to be effective and efficient in producing desirable mutants. Since they brought out less biological damage, the most efficient and effective doses found in gamma irradiation and EMS were the lowest (250 Gy and 10 mM). Comparing both the mutagens, gamma rays are more effective and efficient due to less biological damage and a high mutation rate. These mutagens could be used in breeding programmes to obtain agronomically beneficial mutants, which, on further selection and advancements, could be recommended to develop cultivars with economic benefits. The selected mutants can also be used to study the genes (QTL/SNPs) governing desirable traits like stress tolerance, physiological and agronomic traits and their functions to aid in advanced breeding procedures.

Acknowledgements

This work was supported by Department of Biotechnology-Junior Research Fellowship (DBT-JRF), Government of India. Authors are thankful to ICAR-National Research Centre for Banana, Tiruchirappalli, Tamil Nadu, India for providing facilities for gamma irradiation.

Authors' contributions

MS conducted field experiment, collected data, statistical analysis and manuscript drafting, CV Framing out the research work, Designing the experiment, Manuscript editing, MV Manuscript editing, KS Manuscript editing, TR Guided in conducting field experiments, SM Guided in conducting field experiments. All authors read and approved the final manuscript

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Supplementary data

Supplementary Table 1. Biological damages caused by the physical and chemical mutagens on TRY 1 finger millet

Supplementary Table 2. Frequency of chlorophyll mutants in M_2 generation of different mutagens

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