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Effect of Chemical Mutagen on Growth Parameters of Foxtail Millet (*Setaria italica* L.) in M1 Generation

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ABSTRACT

Background: In the modern day, breeding techniques that use physical and chemical mutagens to induce mutation in a variety of plants are quite effective in increasing the crop's economic worth through genetic alteration.

Methods: Foxtail millet [*Setaria italica* (L.) Beauv.] seeds were subjected to a range of ethyl methane sulphonate (EMS) concentrations, from 0.2% to 0.9%, to produce genetic variability in the current study. The data were recorded in germination paper, pot culture and field conditions for genetic variations.

Result: The mutant 1 (M1) generation of foxtail millet showed differences in seed germination percentage seedling height, seedling damage and plant survival at maturity. The study revealed that 0.2% EMS was the optimal dose for inducing desirable mutations, achieving high seed germination (87.96%), minimal seedling injury (5.49%) and enhanced survival (83.73%). This treatment improved key traits like productive tillers, flag leaf length and panicle length, while reducing plant height (7.10 cm), favouring early maturity. Higher EMS doses (0.3% and 0.4%) induced excessive injury (30.34% and 50.19%) and reduced survival, making them less effective. Overall, 0.2% EMS demonstrated superior mutagenic efficiency and effectiveness, offering potential for enhancing desirable traits in foxtail millet breeding.

Key words: EMS, Foxtail millet, Mutagen, Seedling injury, Survival percentage.

INTRODUCTION

Millets are often referred to as "Nutri cereals" due to their high nutritional value. Among them, foxtail millet is particularly rich in proteins, dietary fibres, calcium and vitamins. Foxtail millet has a low glycemic index and is rich in phytochemicals and phenolic compounds, offering numerous health benefits (Goudar and Sathisha, 2016; Yang *et al.,* 2022; Mal *et al.,* 2010). Consumption helps lower blood glucose and cholesterol levels (Ghimire *et al.,* 2018, Kamatar *et al.,* 2015). The United Nations declared 2023 as the "International Year of Millets" to emphasize its importance in food security and sustainability (FAO, 2012). Yang *et al.* (2012) identified foxtail millet as the second-most productive millet globally, following pearl millet. It is widely cultivated in warm and temperate climates due to its resistance to heat, stress and drought.

As a Poaceae crop, foxtail millet (*Setaria italica* L.) is predominantly self-pollinating, with a chromosome number of 2n = 2x = 18 and a low cross-pollination rate of 4% (Li *et al.,* 1935). It features a spike-like inflorescence with numerous spikelets and bristles, where one flower is sterile and the other is bisexual. The bisexual flower has two long styles with brushy stigmas and three stamens. Malm and Rachie (1971) reported that anthesis typically occurs between midnight and morning, depending on environmental factors.

Additionally, its anti-pest properties make it less susceptible to diseases, allowing for cultivation with minimal concerns (Rajasekaran and Francis, 2020). With a shorter growing season than wheat and rice, foxtail millet is widely produced in India, China, Europe, Africa and America. In India, it is cultivated in Andhra Pradesh, Karnataka, Tamil Nadu, Uttar Pradesh, Uttarakhand, Maharashtra, Rajasthan, Gujarat and the North Eastern states (Saini *et al.,* 2021). Enhancing its morphological, physiological and genetic traits is essential for improving yield and performance.

Since yield is a crucial factor in agriculture, assessing genetic variability, heritability and genetic advancement is necessary. Foxtail millet (*Setaria italica*) is emerging as a model plant due to its short stature, rapid life cycle, high photosynthetic efficiency, self-compatibility, true diploid nature, high seed production, small genome size and stress resistance. However, its genetic research lags other model plants like maize, rice and Arabidopsis. Green foxtail (*Setaria viridis*), the ancestor of foxtail millet, is gaining

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attention as an alternative model due to its short stature, quick life cycle and genetic attributes (Doust *et al.,* 2009; Li and Brutnell, 2011; Huang *et al.,* 2016; Pant *et al.,* 2016).

Unlike *Setaria viridis*, *Setaria italica* has received limited research focus. While mutant resources exist for *Setaria viridis* (Brutnell *et al.,* 2010; Huang *et al.,* 2016), only a few reports are available for *Setaria italica* (Gupta and Yashvir, 1976). Genetic variation plays a crucial role in crop improvement. In traditional plant breeding programs, such variation is typically created through hybridization, followed by selection from the segregating generations (Sebastian *et al.,* 2025). In such scenarios, mutation breeding serves as an effective alternative. Both radiation and chemical mutagens have proven useful in generating mutations and promoting genetic recombination, ultimately contributing to greater variability in traits governed by quantitative inheritance (Saikia *et al.,* 2025). Hence, The primary objective of the present research is to generate genetic variations and differences for crop enhancement through induced mutation in the locally cultivated foxtail millet variety through chemical mutagen EMS.

MATERIALS AND METHODS

The locally cultivated variety of foxtail millet was used for inducing mutation and creating variability. The chemical Ethyl methane sulphonate (CH3SO2OC2H5), with a molecular weight of 124 (Sigma Chemical Co. Ltd. USA) was used as mutagen.

Treatment with the mutagen

Dry, healthy and uniform seeds of the local variety with a moisture content of 10-12% were soaked in distilled water for six hours before being treated with a freshly prepared aqueous solution of ethyl methane sulphonate at varying concentrations (0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9%) for six hours. The same set of 150 seeds per concentration was then soaked in distilled water and used as a control. The treated seeds were raised in germination paper, pots and field condition.

LD_{50} dose to raise M1 generation

The lethal dose (LD_{50}), appropriate EMS concentrations and seed treatment period were all determined by raising the treated seeds in germination paper, pots and in field condition. LD_{50} is the dosage of the mutagen where 50% of individual will survive.

After being immersed in distilled water for seven or six hours, the chosen seeds (M0) were treated for seven or six hours with varying doses of EMS. The control group consisted of the untreated seeds. 150 seeds were utilized for each treatment. To remove any remaining pesticides, the treated seeds were thoroughly cleaned with tap water for an hour. Fifty seeds from each treatment were used for laboratory seed germination.

To record seed germination and seedling height on the seventh day, three replications, each containing ten seeds, were maintained in petri dishes using seed germination paper. The M1 generation was raised in the field using the 150 treated seeds that remained from each treatment.

The field experiments were conducted in the Department of Crop Improvement experimental plot, SRMCAS, Chengalpattu. The experimental field's soil type was fine, well-drained and quite deep. The average annual rainfall was 500 mm, while the average minimum temperature was 21.52°C and the maximum was 35.70°C. The RBD design was followed for conducting the studies. There were fifty plants in each plot. Two rows and two plants were separated by 45 by 30 cm.

Observations recorded in M1 generation

The percentage of seeds that germinated was determined by counting the number of seeds that displayed the radical and plumule emergence. Five seedlings from each treatment and control were chosen at random on the seventh day of seeding to measure the length of the roots and shoots. Seedling injury was defined as a decrease in the mean seedling length relative to the control and was represented as a percentage.

$$\text{Seedling injury (\%)} = \frac{(\text{Control seedling height} - \text{Treatment seedling height})}{\text{Control seedling height}} \times 100$$

Survival rate (%) =

The percentage of plants that reached maturity (45 days)

Cultivation practices of *S. italica* was followed as explained elsewhere (Gupta and Yashvir, 1976). Observations were recorded on five randomly selected plants from each treatment. Data were collected for various traits, including germination percentage, root length, shoot length, seedling injury, seedling survival, days to 50% flowering, plant height, number of productive tillers, single plant yield and 1000-seed weight.

Statistical analysis

Fisher's LSD (Least significant difference) was used as a post hoc tests to ascertain significant differences among treatments at p=0.05. The data were summarized as the means of three replicates with standard de*via*tion as the measures of variability. A one-way ANOVA test was conducted to determine significant differences due to various treatments. The R Studio 4.4.1 application was used to perform statistical analysis and create graphical data presentations.

RESULTS AND DISCUSSION

The assessment of genetic variability among the studied traits revealed differences in the extent of variation, heritability and genetic advance, which are crucial for determining the effectiveness of selection in a breeding program. For every attribute under study, a broad range of variance was noted within the M1 generation.

Optimization of mutagen (LD_{50} value) based on germination %, root length, shoot length, seedling height and seedling injury

Germination %

Seed germination is an important criterion to fix the LD_{50} *i.e.,* optimum dose to create mutation. It was observed that the mutagenic treatment treatments positively affected seed germination ranging from 87.96% to 56.07% and showed highest decline in germination (56.07%) in 0.4%. Francis *et al.* (2022) also reported that variable doses of EMS are inversely proportional to the germination percentage.

Effect of mutagen on root length, shoot length, seedling height and seedling injury

LD_{50} was optimized based on the reduction in root length, shoot length, seedling height and seedling injury. Reduction in height (4.35 cm) favouring early maturity was noticed in 0.2% EMS than control (8.8 cm) which was found to be on par with earlier report (Anittha and Mullainathan, 2018). It was observed that the increased dose of mutagen caused injuries to the seedling and resulted in death of the seedlings than the minimal doses and control. The earlier report of Anittha and Mullainathan (2018) were same as 50.19% (0.4 EMS), 30.34%) (0.3% EMS) seedling injury in present study. As a result of lower injury levels, the seedling survival percentage increased as the EMS dosage decreased, ranging from 53.19% at higher doses to 83.73% at 0.2% EMS. These findings align with previous studies in Horse gram by Awte and Bolbhat (2014), Barnyard millet by Bolbhat and Bhalekar (2020) and Ramesh *et al.* (2019) and finger millet by Bolbhat and Thikekar (2020), where lower mutagenic doses enhanced survival, while higher doses reduced plant viability. The data is given in a bar diagram in Fig 1.

Mutagenic effectiveness and mutagenic efficiency

Mutagenic effectiveness represents the cultivars response or mutation rate of cultivar to the increase in concentration of the mutagen. Mutagenic effectiveness was highest at 0.2% EMS and lowest at 0.6% EMS. This dosage has created observable variation in seedling height, productive tillers, flag leaf length and panicle length promoting for further selection process because of high heritability and genetic advance. Unlike higher EMS doses that compromised plant *via*bility, 0.2% EMS maintained a favourable balance, achieving beneficial genetic changes without excessive harm. Overall, 0.2% EMS proved to be the optimal treatment due to its ability to induce desirable mutations effectively and efficiently. By enhancing key agronomic traits while minimizing biological damage, 0.2% EMS offers the greatest potential for improving foxtail millet through selection and breeding strategies.

Mutagenic efficiency considers the biological damage for the mutations detected. The highest mutagenic efficiency at 0.2% showed highest germination rate (87.96%), minimal seedling injury (5.49%) by giving minimum stress to the plant, highest survival rate (83.73%) outperforming both the control (79.25%) and all other mutagenic treatments. In contrast, higher EMS concentrations, such as 0.3% and 0.4% EMS, induced severe seedling injury (30.34% and 50.19%, respectively) and drastically reduced plant survival, highlighting their lower efficiency. The ability of 0.2% EMS to induce positive changes with minimal damage underscores its superior mutagenic efficiency.

The trait days to 50% flowering exhibited low phenotypic and genotypic variation, with a PCV of 5.08% and a GCV of 1.02%, respectively. Negative heritability and minimal genetic advance as a percentage of the mean (-0.42%) indicate that this trait is predominantly influenced by environmental factors, making selection ineffective for improving this character. Similarly, days to maturity showed limited variability (PCV = 5.02%, GCV = 1.77%) with low heritability (0.12) and modest genetic advance (1.29%), further suggesting that selection may not be effective in enhancing this trait.

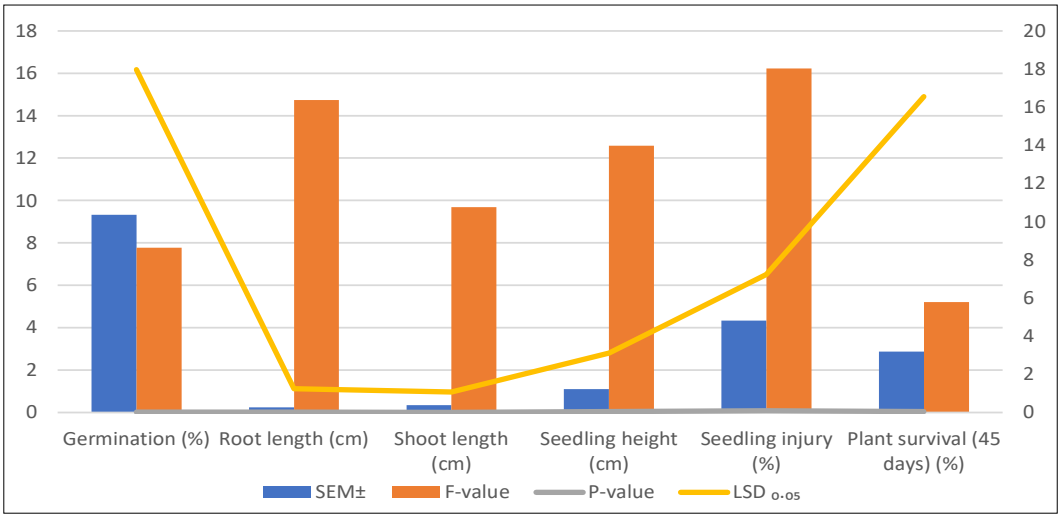


Fig 1: Effect of EMS on the root length, shoot length, seedling height, seedling injury and plant survival.

Table 1: Effect of mutagens on growth parameters in M1 generation of foxtail millet.

Treatments	Days to 50% flowering	Days to maturity	Plant Height (cm)	Number of productive tillers	Flag leaf length (cm)	Flag leaf width (cm)	Panicle length (cm)	1000 grain weight (g)
0.2%	52.05±3.82	80.35±6.98	143.11±23.26	2.47±0.46	23.52^{b}±0.59	1.42±0.18	17.86^{ab}±2.38	2.51±0.37
0.3%	54.39±3.07	82.85±5.16	146.87±13.58	2.33±0.61	31.93^{a}±4.01	1.55±0.08	14.85^{c}±1.67	2.81±0.15
0.4%	54.83±3.65	86.85±2.85	154.47±8.11	3.40±0.53	27.90^{ab}±2.43	1.58±0.14	14.47^{c}±2.18	2.55±0.27
0.5%	53.73±2.83	86.85±0.69	158.07±20.67	2.47±0.50	29.47^{a}±2.69	1.55±0.20	15.19^{bc}±0.86	2.74±0.30
0.6%	52.24±3.26	85.99±3.00	160.4±1.56	3.07±0.42	27.87^{ab}±2.80	1.64±0.14	19.51^{a}±2.01	2.51±0.24
0.7%	56.34±1.92	89.43±2.77	173.73±6.27	2.2±0.53	30.93^{a}±1.36	1.41±0.10	17.03^{abc}±1.41	3±0.19
0.8%	55.53±0.82	84.63±1.10	157.17±8.77	3.13±0.42	30.10^{a}±1.01	1.48±0.11	15.43^{bc}±1.83	2.59±0.22
0.9%	55.17±2.45	84.85±5.38	161.16±14.97	2.53±0.50	31.60^{a}±2.08	1.63±0.12	15.90^{bc}±1.04	2.93±0.31
F value	0.88	1.43	2.13	2.73	3.62	1.14	3.03	1.56
P value	0.54	0.27	0.11	0.08	0.02	0.39	0.04	0.23
LSD 0.05	NS	NS	NS	NS	4.38	NS	3.01	NS

Effect of mutagen on the yield and yield parameters in M1 generation

Using statistical parameters like mean, range and genetic parameters estimated by Panse and Sukhatme (1985) methodology, the various mutagenic treatment populations in M1 generation were assessed to determine the type and extent of induced polygenic variability in traits like plant height (cm), number of productive tillers/plants and grain yield per plant (g). Data in regards to mean performance and genetic parameters are given in Table 1.

Plant height

Plant height was significantly lower at 0.2 (158.22 cm) than in all mutant populations. While other dosages of increased height show a positive shift from the mean, the decrease in plant height in 0.2% and 0.3% suggests a negative shift. Negative shift in plant height is preferred for semi-dwarf and intermediate varieties. To identify plants with reduced height, the population's dwarf individuals must be isolated. Plant height displayed moderate variability with a PCV of 8.30% and a GCV of 4.33%. The low heritability estimate (0.27) and genetic advance (4.67%) indicate that environmental factors significantly influenced this trait, making selection for plant height less reliable for genetic improvement. This was in support with the findings of Francis *et al.* (2022).

Number of productive tillers per plant

The number of productive tillers per plant varied significantly due to the induced mutation. A noticeable increase in tiller number was observed after EMS treatment, with the highest number recorded at 0.2% EMS. This treatment showed a phenotypic coefficient of variation (PCV) of 21.93% and a genotypic coefficient of variation (GCV) of 12.29%, along with moderate heritability (0.31) and a genetic advance of 14.19% indicating potential for selection.

Flag leaf length and width

Flag leaf length and width favours increased length of the panicle, size of the spikelets and exertion of panicle. This study expressed moderate variability (PCV = 11.73%, GCV = 8.01%) with moderate heritability (0.46) and a genetic advance of 11.26% whereas flag leaf width had minimal variability (PCV = 9.55%, GCV = 2.06%) and extremely low heritability (0.04) with a negligible genetic advance (0.91%), making selection for this trait ineffective.

Panicle length

The trait panicle length showed moderate genetic variability, with a PCV of 13.67% and a GCV of 8.69%. With moderate heritability (0.40) and a genetic advance of 11.38%, selection could moderately improve this trait.

Grain yield per plant and 1000 grain weight

Among the population, the highest grain yield (16.49 g) and 1000 seed was was recorded in 0.2% EMS with high heritability and genetic advance whereas for 1000-grain weight, although the PCV was 10.74%, the GCV was

considerably lower at 4.26%, with low heritability (0.15) and a genetic advance of 3.48%, indicating that selection may have limited success in enhancing this trait.

Hence, 0.2% EMS has improved seed germination (87.96%), better seedling growth and minimal seedling injury (5.49%), indicating enhanced tolerance to mutagenic stress and ideal for selection of traits like the number of productive tillers, flag leaf length and panicle length. with a height of 7.10 cm with higher survival percentage. Higher EMS concentrations, such as 0.3% and 0.4%, caused excessive injury and reduced plant viability, making them less suitable for selection.

CONCLUSION

Mutation breeding is a potent strategy used in crop improvement programs. As foxtail millet is a self-pollinating crop with limited genetic variation, the development and field phenotypic characterization of an EMS-induced mutant population offers a *via*ble approach to enhance its genetic diversity. The current study indicates lower doses of mutagen is inversely proportional to the effect on the yield and yield parameters in M1 generation. Having this as base, the high frequency of beneficial mutations seen in the M1 generation will be assessed in subsequent generations and applied to the production of new varieties.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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