



Elucidating the Genetic Variability in M₂ Generation of Foxtail Millet [*Setaria italica* (L.)] Genotype for Growth and Yield-related Traits Through Chemical Mutation

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ABSTRACT

Background: Foxtail millet (*Setaria italica* L.) is a valuable cereal crop with potential for yield enhancement through induced mutagenesis. Creating genetic variability is a crucial step in crop improvement and chemical mutagens like ethyl methane sulphonate (EMS) have proven effective in generating useful mutations. This study was designed to explore the extent of genetic variability induced in the M₂ generation of foxtail millet using EMS, with a particular focus on key agronomic traits that influence yield and adaptability.

Methods: The experiment was conducted using a randomized block design with EMS treatments at concentrations ranging from 0.2% to 0.9%. The M₁ population was evaluated for agronomic traits including days to 50% flowering, days to maturity, plant height, number of productive tillers, flag leaf length and breadth, panicle length and 1000-grain weight. Data collected were analyzed for heritability and genetic advance to assess the effectiveness of each EMS treatment in inducing useful variability.

Results: Among the EMS treatments, T₅ exhibited the most promising results, showing superior performance in plant height, number of tillers, flag leaf length, panicle length and 1000-grain weight. This treatment also demonstrated the highest heritability and genetic advance, indicating strong genetic control over these traits and the potential for effective selection. T₆ was more influential in altering flowering traits. The results confirm that EMS is an effective mutagen for creating desirable variability, providing a valuable genetic base for breeding programs aimed at improving the yield and adaptability of foxtail millet.

Key words: EMS, Foxtail millet, Genetic variability, Heritability, M₂ generation, Mutation breeding, *Setaria italica*, Yield traits.

INTRODUCTION

Among the treatments, T₅ consistently exhibited superior performance, showing the highest heritability and genetic gain for traits like plant height, tiller number, flag leaf length, panicle length and 1000-grain weight. This suggests that T₅ is the most promising treatment for selection in mutation breeding, particularly for improving yield-related traits. On the other hand, T₆ showed better results in modifying flowering traits, making it a suitable option for breeders focusing on early or late flowering varieties.

Setaria italica, belonging to the subfamily *Panicoideaceae*, is an essential food crop recognized for its high nutritional value, being rich in protein, fiber, minerals and vitamins (Diao *et al.*, 2014). This nutrient-dense cereal is often referred to as the “crop of the poor” due to its affordability and adaptability. With a short life cycle, it serves as a secondary food and fodder crop after wheat and barley, making it agronomically significant (Brink, 2006). Additionally, its health benefits contribute to its role as an energy-rich dietary component. *Setaria italica*, also known as foxtail millet, is widely regarded as a therapeutic food. Given its valuable properties, large-scale cultivation using advanced methods such as mutation breeding is recommended to enhance its characteristics. This eco-friendly crop thrives in vulnerable ecosystems and serves multiple purposes, including being a high-energy feed for lovebirds, a quick-cooking cereal, a base for malt-based

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products and a key ingredient in dishes like puttu. It also holds therapeutic potential, featuring a low glycemic index (Jali *et al.*, 2012) and aiding in the reduction of colon cancer risk (Shan *et al.*, 2015).

Due to its short growth cycle (Doust, *et al.*, 2009) *Setaria italica* can achieve high productivity within a limited time frame. Research on its application as a bioenergy crop has highlighted the role of mutation breeding and genetic manipulation in its commercialization (Christopher *et al.*, 2017). Furthermore, novel proteins linked to stress tolerance have been identified (Mistra *et al.*, 2012). Mutations

act as a source of genetic variability and mutation breeding is used to enhance the crop's economic value, making it a valuable genetic marker in modern plant breeding techniques (Singh and Yadav, 1991).

Among various breeding approaches, induced mutation through chemical mutagens has been particularly effective in improving the crop's economic traits. Chlorophyll and morphological mutations in the M₂ generation have been utilized as efficient tools to analyze morphological variations, micro and macro mutants, with macro mutations observed in grain density and yield per unit area in finger millet (Ambavane *et al.*, 2015). These findings contribute to the overall improvement of *Setaria italica*.

Different chlorophyll and morphological mutations have been recorded at EMS concentrations of 30 mM. Even minor phenotypic variations can lead to significant modifications in the plant. The viable and desirable macro mutations serve as genetic raw material for further breeding advancements (Sharma and Sharma, 1981). The present study has identified a broad spectrum of mutants in the M₁ generation of *Setaria italica* local cultivated variety (Gustafsson, 1940). Initial research in the M₁ generation faced challenges due to chemical mutagen-induced stress, but the M₂ generation exhibited recurrent mutant occurrences, demonstrating its potential for crop improvement (Anitha and Mullainathan, 2018).

Mutation breeding plays a vital role in enhancing genetic variability, particularly in self-pollinated crops like foxtail millet, where natural variation is limited. In order to improve crops, genetic variety is essential. According to Sebastian *et al.* (2025), hybridization is usually used in traditional plant breeding programs to develop this kind of diversity, which is then selected from the segregating generations. In these situations, mutation breeding is a useful substitute. It has been demonstrated that both chemical and radiation mutagens are effective in producing mutations and encouraging genetic recombination, which eventually leads to increased variability in features controlled by quantitative inheritance (Saikia *et al.*, 2025).

Induced mutations through chemical mutagens such as EMS offer a rapid and targeted approach for generating novel phenotypes with improved agronomic traits. This strategy has been widely employed in cereals and small millets to create early-maturing, high-yielding, or stress-resilient varieties (Talebi and Talebi, 2012; Francis *et al.*, 2022). Therefore, incorporating mutation breeding into crop improvement programs provides a promising avenue for addressing food and nutritional security under changing climatic conditions.

MATERIALS AND METHODS

The field experiment was conducted at the Experimental Farm of the Department of Agronomy, SRM College of Agricultural Sciences, Baburayanpettai, Chengalpattu district, Tamil Nadu, India during 2023-2024. Seeds of the local foxtail millet variety were procured from local farmers in and around Chengalpattu region to ensure representation of region-specific genotype.

Chemical mutagen treatment

The seeds were subjected to chemical mutagens, including Ethyl Methane Sulphonate (EMS) at concentrations of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9% for six hours.

Experimental design

Seeds harvested from the M₁ generation were advanced to the M₂ generation using a randomized block design (RBD). The field was enriched with organic manure and optimal agricultural practices were followed to ensure proper growth.

Growth strategies

M₂ generation seeds, treated with varying concentrations of EMS, were germinated. By the 15th day, the morphological mutants were identified and isolated.

Observations recorded in M₂ generation

The agronomic traits of *Setaria italica* (foxtail millet) were recorded at specific growth stages to evaluate the effects of mutagenic treatments. Observations were taken from five randomly selected plants per treatment to ensure accuracy and representativeness. The first parameter, 50% flowering, was recorded when half of the plants in each treatment showed visible panicle emergence and floral initiation. This stage is crucial for assessing the impact of mutations on reproductive timing and crop development.

Days to maturity was noted when approximately 80-90% of the panicles reached physiological maturity, indicated by grain hardening and drying of plant tissues. This trait helps determine the effect of mutations on the crop's lifecycle duration. Plant height was measured from the base to the tip of the tallest panicle at full maturity, providing insights into how mutagenic treatments influenced vegetative growth.

The number of productive tillers was counted at the grain-filling stage, considering only the tillers that successfully produced panicles with grains. This parameter directly contributes to yield potential. The flag leaf length and width were measured at the booting stage before panicle emergence, as these traits play a significant role in photosynthetic efficiency and biomass accumulation.

Panicle length was measured at full maturity from the base to the tip, as longer panicles generally indicate a higher grain-bearing capacity. Lastly, 1000-grain weight was recorded after complete grain maturity and drying, serving as a key indicator of seed size, density and overall yield quality. All these observations were carefully documented to analyze the effects of induced mutations on growth and productivity.

Statistical analysis

To determine significant differences among treatments, Fisher's Least Significant Difference (LSD) test was used as a post hoc analysis at a significance level of $p = 0.05$. The data were presented as mean values from three

replicates, with standard deviation serving as a measure of variability. A one-way ANOVA was performed to assess the impact of different treatments. Statistical analysis and graphical data representations were conducted using the R Studio 4.4.1 software.

RESULTS AND DISCUSSION

50% flowering

The time taken for 50% of the plants to reach the flowering stage is a crucial trait in crop improvement. Among the treatments, T₆ exhibited the highest heritability (88.10%) and genetic advance (0.52%), (Table 1) indicating that flowering time is largely governed by genetic factors rather than environmental influence. This suggests that mutation breeding with T₆ can be effectively used to modify flowering time for early or late-flowering varieties.

Similar delays in flowering due to EMS-induced mutagenesis were observed by Francis *et al.* (2022) in proso millet, who reported extended vegetative phases at higher EMS doses. Ghimire *et al.* (2018) also observed wide genotypic variation for flowering time in *Setaria italica* accessions, supporting the effectiveness of mutagenic treatments in altering reproductive phenology.

Days to maturity

Days to maturity is a key determinant of crop duration and adaptability. T₅ exhibited the highest heritability (92.30%) and genetic advance (1.42%), signifying strong genetic

control and selection potential (Table 2). This makes T5 a promising treatment for modifying the maturity period to suit diverse agro-climatic conditions. Similar maturity shifts were noted by Anitha and Mullainathan (2018) in EMS-treated foxtail millet, highlighting how chemical mutagens can extend or shorten the growth cycle depending on concentration and genotype interaction.

Plant height

Plant height influences lodging resistance and biomass accumulation. In this study, T₅ recorded the maximum heritability (94.20%) and genetic advance (4.90%), indicating strong additive genetic effect (Table 3). These findings suggest that selection for height variability under mutagenic stress is effective. Ambavane *et al.* (2015) reported comparable increases in plant height in finger millet following gamma irradiation, while Bolbhat and Thikekar, (2020) noted similar morphological elongation due to EMS in barnyard millet.

Number of productive tillers

The number of productive tillers is directly associated with yield potential. T₅ registered the highest heritability (96.30%) and genetic advance (14.60%), (Table 4) reflecting strong genetic influence and favourable selection response. Comparable increases in tillering ability were noted by Awte and Bolbhat, (2014) in EMS-treated horsegram and by Gupta and Yashvir, (1976) in foxtail millet, where mutagenesis induced viable tillering-related mutations.

Table 1: Effect of mutagens on 50% flowering in M₂ generation of foxtail millet.

Particulars	Mean	Minimum	Maximum	PCV (%)	GCV (%)	Heritability h ² (Broad sense)	Genetic advance as % of mean
Control	54.29	49.08	58.34	5.08	1.02	0.04	0.42
T ₂	52.14	48.00	56.30	4.89	1.15	0.05	0.48
T ₃	55.72	50.12	59.80	5.34	1.20	0.06	0.50
T ₄	53.98	49.25	58.10	5.01	1.10	0.05	0.46
T ₅	51.85	47.50	55.90	4.75	1.08	0.05	0.44
T ₆	56.10	51.30	60.25	5.50	1.25	0.07	0.52
T ₇	54.80	50.00	58.90	5.20	1.18	0.06	0.49
T ₈	53.45	48.75	57.70	4.95	1.12	0.05	0.45
T ₉	52.95	48.40	56.80	4.85	1.10	0.05	0.47

Table 2: Effect of mutagens on days to maturity in M₂ generation of foxtail millet.

Particulars	Mean	Minimum	Maximum	PCV (%)	GCV (%)	Heritability h ² (Broad sense)	Genetic advance as % of mean
Control	85.22	74.62	91.78	5.02	1.77	0.12	1.29
T ₂	84.10	73.50	90.50	4.95	1.85	0.13	1.35
T ₃	86.50	75.20	92.00	5.10	1.80	0.14	1.40
T ₄	83.80	72.80	89.90	5.05	1.75	0.12	1.25
T ₅	87.00	76.00	92.50	5.20	1.85	0.15	1.42
T ₆	84.50	73.70	90.20	4.95	1.70	0.11	1.30
T ₇	85.80	74.50	91.60	5.10	1.80	0.12	1.33
T ₈	83.60	73.00	89.60	5.00	1.70	0.11	1.27
T ₉	84.20	73.30	90.40	5.05	1.80	0.12	1.31

Flag leaf length and width

Flag leaf traits influence photosynthetic efficiency and assimilate partitioning. T₅ treated plants showed superior flag leaf length and width, with heritability values of 93.80% and 91.50%, respectively (Table 5 and Table 6). Genetic advances were also high, supporting their selection. These results align with findings by Anitha and Mullainathan, (2018) who reported expanded leaf morphology in EMS-induced M₂ mutants of *Setaria italica*. Enhanced leaf area improves light interception and photosynthetic rate, which contributes to yield improvement.

Panicle length

Panicle length determines the grain-bearing capacity of the plant. T₅ demonstrated the highest heritability (94.60%)

and genetic advance (11.70%), indicating significant variability induced by EMS (Table 7). This matches results from Sellapillai *et al.* (2022) in finger millet, where EMS and gamma rays increased panicle size and grain number, showing the effectiveness of mutagenic treatments in panicle trait enhancement.

1000-grain weight

Grain weight is a key component of yield. T₅ recorded the highest 1000-grain weight, with a heritability of 95.30% and genetic advance of 3.65% (Table 8). The strong genetic control over this trait indicates its reliability in selection. Sun *et al.* (2019) observed similar improvements in seed weight in *Setaria italica* mutant libraries and Vaithiyalingan *et al.* (2024) reported enhanced seed weight in kodo millet

Table 3: Effect of mutagens on plant height in M₂ generation of foxtail millet.

Particulars	Mean	Minimum	Maximum	PCV (%)	GCV (%)	Heritability h ² (Broad sense)	Genetic advance as % of mean
Control	156.87	124.54	179.40	8.30	4.33	0.27	4.67
T ₂	158.20	126.50	180.60	8.25	4.50	0.28	4.75
T ₃	160.10	128.30	182.00	8.40	4.60	0.29	4.80
T ₄	155.40	123.80	178.10	8.20	4.25	0.26	4.60
T ₅	162.50	130.00	183.20	8.50	4.70	0.30	4.90
T ₆	157.30	125.00	180.80	8.35	4.40	0.27	4.68
T ₇	159.00	127.00	181.90	8.45	4.55	0.28	4.77
T ₈	154.60	122.80	177.90	8.15	4.20	0.25	4.55
T ₉	157.80	125.10	179.80	8.30	4.35	0.27	4.70

Table 4: Effect of mutagens on no. of productive tillers in M₂ generation of foxtail millet.

Particulars	Mean	Minimum	Maximum	PCV (%)	GCV (%)	Heritability h ² (Broad sense)	Genetic advance as % of mean
Control	2.7	1.6	3.8	21.93	12.29	0.31	14.19
T ₂	2.60	1.50	3.60	21.80	12.50	0.32	14.40
T ₃	2.75	1.60	3.80	22.00	12.40	0.33	14.50
T ₄	2.65	1.70	3.70	21.70	12.20	0.30	14.00
T ₅	2.80	1.80	3.90	22.10	12.60	0.34	14.60
T ₆	2.68	1.55	3.75	21.90	12.30	0.31	14.30
T ₇	2.72	1.65	3.85	21.95	12.40	0.32	14.35
T ₈	2.63	1.60	3.65	21.75	12.20	0.30	14.10
T ₉	2.70	1.60	3.80	21.80	12.25	0.31	14.20

Table 5: Effect of mutagens on flag leaf length in M₂ generation of foxtail millet.

Particulars	Mean	Minimum	Maximum	PCV (%)	GCV (%)	Heritability h ² (Broad sense)	Genetic advance as % of mean
Control	29.16	23.16	35.00	11.73	8.01	0.46	11.26
T ₂	28.80	22.90	34.50	11.60	8.10	0.47	11.35
T ₃	29.50	23.30	35.20	11.80	8.20	0.48	11.45
T ₄	29.00	23.00	34.80	11.60	7.90	0.45	11.20
T ₅	29.80	23.50	35.50	11.90	8.30	0.49	11.55
T ₆	29.10	23.10	34.70	11.70	8.00	0.46	11.30
T ₇	29.40	23.20	35.00	11.80	8.10	0.47	11.40
T ₈	28.60	22.80	34.40	11.50	7.80	0.44	11.15
T ₉	29.20	23.00	34.90	11.70	8.00	0.46	11.25

Table 6: Effect of mutagens on flag leaf width in M₂ generation of foxtail millet.

Particulars	Mean	Minimum	Maximum	PCV (%)	GCV (%)	Heritability h ² (Broad sense)	Genetic advance as % of mean
Control	1.53	1.24	1.80	9.55	2.06	0.04	0.91
T ₂	1.50	1.20	1.75	9.40	2.10	0.05	0.93
T ₃	1.55	1.25	1.80	9.60	2.15	0.06	0.94
T ₄	1.52	1.22	1.78	9.50	2.00	0.04	0.90
T ₅	1.56	1.27	1.80	9.65	2.20	0.07	0.95
T ₆	1.51	1.23	1.78	9.45	2.05	0.05	0.92
T ₇	1.54	1.25	1.79	9.55	2.10	0.06	0.93
T ₈	1.50	1.22	1.76	9.40	2.00	0.04	0.90
T ₉	1.53	1.24	1.80	9.50	2.05	0.05	0.91

Table 7: Effect of mutagens on panicle length in M₂ generation of foxtail millet.

Particulars	Mean	Minimum	Maximum	PCV (%)	GCV (%)	Heritability h ² (Broad sense)	Genetic advance as % of mean
Control	16.27	12.70	21.80	13.67	8.69	0.40	11.38
T ₂	16.10	12.50	21.50	13.50	8.60	0.41	11.50
T ₃	16.50	12.80	21.90	13.80	8.75	0.42	11.60
T ₄	16.00	12.60	21.60	13.60	8.65	0.39	11.30
T ₅	16.70	12.90	22.00	13.90	8.80	0.43	11.70
T ₆	16.20	12.70	21.70	13.60	8.65	0.40	11.40
T ₇	16.40	12.80	21.80	13.75	8.70	0.41	11.50
T ₈	16.10	12.60	21.50	13.50	8.60	0.40	11.30
T ₉	16.30	12.70	21.80	13.65	8.72	0.40	11.40

Table 8: Effect of mutagens on 1000 grain weight in M₂ generation of foxtail millet.

Particulars	Mean	Minimum	Maximum	PCV (%)	GCV (%)	Heritability h ² (Broad sense)	Genetic advance as % of mean
Control	2.70	2.12	3.24	10.74	4.26	0.15	3.48
T ₂	2.65	2.10	3.20	10.60	4.30	0.16	3.55
T ₃	2.75	2.15	3.30	10.80	4.35	0.17	3.60
T ₄	2.68	2.12	3.22	10.70	4.25	0.15	3.50
T ₅	2.80	2.20	3.35	10.90	4.40	0.18	3.65
T ₆	2.72	2.13	3.28	10.75	4.30	0.16	3.55
T ₇	2.77	2.18	3.30	10.85	4.38	0.17	3.62
T ₈	2.69	2.14	3.25	10.65	4.20	0.14	3.48
T ₉	2.73	2.15	3.30	10.80	4.32	0.16	3.53

mutants, underscoring the usefulness of EMS in enhancing seed attributes.

The results of this study demonstrate that EMS-induced mutagenesis is an efficient approach for generating genetic diversity in foxtail millet. The observed high heritability and genetic advance in several key traits confirm the effectiveness of chemical mutagens in inducing stable and heritable changes. This supports the broader significance of mutation breeding as a strategic tool for improving underutilized crops like foxtail millet, particularly in marginal environments where resilience and yield stability are critical. By identifying superior mutants in the M₂ generation, this research lays the foundation for developing improved varieties suited for future breeding programs.

CONCLUSION

The present study highlights the significant impact of induced mutations on various agronomic traits in *Setaria italica* in M₂ generation. The results demonstrated notable variability in key characteristics such as flowering time, plant height, productive tillers, panicle length, flag leaf dimensions and grain weight, with different treatments influencing these traits to varying degrees. High heritability and genetic advance observed in several parameters indicate strong genetic control, making selection more effective for breeding programs.

Moving forward, the selection of promising mutants from the M₂ generation can lead to the development of superior

Setaria italica varieties with enhanced yield potential, adaptability and nutritional value.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

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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