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Assessment on Genetic Diversity in Proso Millet (*Panicum miliaceum* L.) Mutants for Yield and Yield Parameters Raised during M_2 Generation

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ABSTRACT

Background: Proso millet (*Panicum miliaceum* L.) is a climate-resilient, nutrient-dense cereal crop with considerable potential in sustainable agriculture. However, its genetic improvement is hindered by a narrow genetic base. Mutation breeding, particularly through chemical mutagenesis, offers a promising approach to induce genetic variability and develop improved genotypes.

Methods: Ethyl methanesulfonate (EMS) was used to induce mutations in proso millet seeds at different concentrations. The M_2 generation derived from EMS-treated seeds was evaluated for ten morphological and yield-contributing traits, including days to 50% flowering, plant height, number of productive tillers, panicle length, leaf length and breadth and 1000-grain weight. The data were analyzed to evaluate the variability caused by the treatments.

Result: Significant phenotypic variability was observed among the M_2 populations for all studied traits. Treatments with 0.5% and 0.75% EMS were particularly effective, showing enhanced values in several agronomic traits compared to control. These treatments led to the identification of promising mutant lines, indicating the potential of EMS mutagenesis for broadening the genetic base and improving yield-related traits in proso millet.

Key words: Agronomic traits, EMS, Induced variability, M_2 generation, Mutation breeding, Proso millet.

INTRODUCTION

Proso millet (*Panicum miliaceum* L.), an ancient cereal crop, is highly adaptable to marginal environments and exhibits excellent drought tolerance. Despite its nutritional benefits and climate resilience, proso millet remains underutilized in modern breeding programs. Conventional breeding in this crop is constrained by a narrow genetic base, making mutation breeding a viable strategy to enhance genetic variability.

Ethyl methanesulfonate (EMS) is a widely used chemical mutagen known to induce point mutations, primarily through guanine alkylation, resulting in base substitutions during DNA replication (Action *et al.,* 2021). EMS has been effectively employed in cereals and millets to generate desirable traits such as early maturity, dwarfism and increased yield components (Kumar *et al.,* 2025).

Previous studies in foxtail millet have demonstrated that EMS-induced mutants exhibit significant phenotypic variation in plant height, flowering duration and grain weight (Action *et al.,* 2021). This study extends that approach to proso millet, aiming to assess the effectiveness of EMS in inducing beneficial mutations in the M_2 generation and identifying superior lines for breeding programs.

MATERIALS AND METHODS

Plant material and treatment details

The current study was conducted at SRM College of Agricultural Sciences. The M_1 generation which was cultivated during 2024 and was harvested to proceed for the next generation. The M_2 generation was grown in the *rabi* season of 2025.

Uniformly healthy seeds of proso millet were pre-soaked in distilled water for 6 hours and then treated with EMS at concentrations of (T_2) 0.1%, (T_3) 0.2%, (T_4) 0.3%, (T_5) 0.4%, (T_6) 0.5%, (T_7) 0.75% and (T_8) 1.0% of 250 seed for each treatment for 6 hours. Treated seeds were thoroughly washed in running water to eliminate residual EMS and sown in the field using a randomized block design

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(RBD) with three replications. Untreated seeds (T_1) served as the control.

The lethal dose (LD_{50}) of the chemical T_7 was determined and established as the fixed dose for subsequent treatments. This fixed LD_{50} dose was applied to the seeds at both pre- and post-exposure stages of M_1 generation. The harvested seeds from 50 randomly selected plants of each treatment were then allowed to grow and mature and the subsequent generations (M_2) were cultivated for further analysis.

Data recorded in M_2 generation

In the M_2 generation, a comprehensive phenotypic evaluation was conducted to assess the mutagenic impact of EMS on agronomic and morphological traits of proso millet. Plants were grown under uniform agronomic conditions with proper spacing and cultural practices to avoid environmental variation. Observations were recorded from ten randomly selected healthy plants per treatment per replication and the mean values were computed for each trait.

The following ten quantitative traits were recorded based on their relevance to plant development, productivity and breeding significance: days to 50% flowering, plant height, number of productive tillers per plant, panicle length (cm), number of leaves per plants, internode length (cm), flag leaf length (cm), flag leaf width (cm), days to maturity, 1000 seed weight. Each trait was carefully recorded using standard procedures to ensure accuracy.

Statistical analysis

The collected data were analyzed statistically to assess the significance of variation among the EMS treatments. The analysis of variance (ANOVA) conducted on these parameters demonstrates the mutagenic efficiency of EMS in generating genetic diversity within the crop. This variability analysis was performed using R Studio version 4.4.1.

RESULTS AND DISCUSSION

Ten agronomic traits were evaluated in eight treatments, presumably mutagenic or varietal, to determine genetic variation, heritability and treatment effects. The data are expressed as means, standard deviations and ANOVA-based significance levels (Table 1), supported by genetic parameters (Table 2). The findings indicate significant genetic variation and differential responses to treatments among traits.

Days to 50% flowering

Days to 50% flowering varied considerably from 46.87 days (T_1) to 58.53 days (T_3). ANOVA revealed that there was a significant treatment difference (F = 5.15; P = 0.004) for which critical LSD was 6.85. Treatments 1, 2 and 7 had significantly earlier flowering, indicating that these treatments are earliness inducers. Genetically, the character was under moderate heritability (0.83) and low genetic progress (10.44%), which denoted partial environmental

Table 1: ANOVA for morphological and yield-contributing traits in M_2 generation of proso millet.

Treatments	50% Flowering	Plant height (cm)	No. of productive tillers	Panicle length (cm)	No of leaves (cm)	Internode length (cm)	Flag leaf length	Flag leaf width	Days to maturity	1000 Grain wt
1	46.87^{b}±3.53	97.20±3.83	10.80±4.01	27.37±1.80	5.73^{abc}±0.64	10.80^{de}±0.17	23.38^{b}±0.75	1.15±0.23	72.93±7.79	2.51±0.57
2	47.33^{b}±3.63	104.00±5.37	10.87±1.81	28.57±0.91	6.00^{ab}±0.20	10.73^{de}±0.45	27.0^{a}±2.70	1.29±0.05	75.67±5.64	2.81±0.40
3	58.53^{a}±0.99	97.40±5.03	8.13±0.50	29.00±0.87	5.73^{abc}±0.50	11.97^{b}±0.67	25.41^{a}±0.85	1.31±0.09	78.40±1.31	2.55±0.70
4	57.07^{a}±8.89	92.00±6.84	8.40±1.06	27.43±0.51	6.00^{ab}±0.35	11.67^{bcd}±0.76	25.52^{a}±1.42	1.29±0.25	80.73±2.84	2.74±0.37
5	57.80^{a}±1.74	99.33±18.68	8.60±0.87	26.80±6.24	5.20^{c}±0.53	12.93^{a}±0.71	26.15^{a}±2.05	1.37±0.13	78.87±3.41	2.51±0.58
6	56.60^{a}±4.16	111.93±9.44	10.33±1.10	28.00±1.06	6.07^{a}±0.70	13.80^{a}±0.46	26.58^{a}±1.94	1.15±0.11	81.13±2.00	3.00±0.55
7	47.93^{b}±5.78	107.20±1.97	11.00±0.40	32.60±1.44	6.47^{a}±0.12	11.07^{cde}±0.21	26.45^{a}±2.52	1.21±0.15	77.27±0.31	2.59±0.65
8	55.67^{a}±0.23	102.27±5.26	11.47±1.30	30.73±1.53	5.27^{bc}±0.31	11.93^{bc}±0.40	26.20^{a}±1.71	1.37±0.15	78.47±3.50	2.93±0.73
F Value	5.15	1.44	1.90	1.82	2.89	13.42	4.09	1.14	1.37	1.56
P value	0.004	0.27	0.15	0.16	0.04	3.09	0.01	0.39	0.29	0.23
LSD 0.05	6.85	NS	NS	NS	0.76	0.88	1.68	NS	NS	NS

impact and probable dominance or epistatic gene action (Kumari *et al.,* 2019).

Plant height

While the height of the plant varied between 92.00 cm and 111.93 cm within treatments, the variations were not statistically significant (P = 0.27). However, genetic analysis revealed high heritability (0.95) and high GA (% mean) of 23.26%, indicating that the variability observed is primarily genetic in origin. Treatment 6 had the highest height of plants, which may be investigated for enhancing biomass or lodging tolerance in future breeding programs (Trivedi *et al.,* 2017).

Number of productive tillers

This character had no significant treatment effect (P = 0.15), even though the highest mean was recorded in Treatment 8 (11.47), closely followed by T_7 and T_2. Genetically, the character was highly heritable (0.96) and had the highest GA (% mean) (25.25%), both supporting its potential for effective selection though at present statistically not significant. The extensive diversity and high genetic progress signify the potential of mutagenesis for improving tiller number (Bedis *et al.,* 2006).

Panicle length

Panicle length ranged between 26.80 cm (T_5) and 32.60 cm (T_7), but no significant variation was noted (P = 0.16). The character was highly heritable (0.91) and had moderate genetic advance (13.01%), suggesting the dominance of additive gene action with potential minor environmental regulation. T_7 recorded the maximum panicle length and should be assessed for its yield potential in further studies (Muduli and Misra, 2008).

Number of leaves

Leaf number was significantly different among treatments (P = 0.04), with Treatment 7 (6.47) and Treatment 6 (6.07) being superior to the rest. The LSD test revealed significant differences at 0.76 units. Genetically, this characteristic revealed high heritability (0.91) and genetic advance (20.60%), reflecting the dominant role of additive genes. Treatments 6 and 7 can therefore be given priority in selection.

Internode length

Internode length was greatly affected by treatment (P = 3.09) and the greatest value was observed in Treatment 6 (13.80 cm) that differed significantly from others (LSD = 0.88). The characteristic had high PCV (12.30%) and GCV (12.09%), heritability (0.96) and genetic advance (24.48%), indicating it as the most suitable character for selection and a target for enhancing plant architecture.

Flag leaf traits (length and width)

Flag leaf length also revealed highly significant treatment differences (P = 0.01). Treatments 2 to 8 were significantly greater than Treatment 1, with the maximum length being for Treatment 2 (27.01 cm). The trait possessed extremely high heritability (0.95) and GA of 17.16%, which favors selection for photosynthetic potential (Dhanalakshmi *et al.,* 2019).

Flag leaf width, however, wasn't significantly different across treatments (P = 0.39), though T_5 and T_8 had the highest values (1.37 cm). Genetic parameters also continue to give support to the utility of selection for the trait due to high heritability (0.92) and GA (15.20%).

Days to maturity

Notwithstanding a variation between 72.93 and 81.13 days, the variation in days to maturity between treatments was statistically not significant (P = 0.29). Genetically, this character had moderate heritability (0.82) and lower GA (9.63%), reflecting lesser gain from selection and greater environmental influence (Muduli and Misra, 2007).

1000-grain weight

Treatment differed in 1000-grain weight from 2.51 g (T_1, T_5) to 3.00 g (T_6), but not statistically significant (P = 0.23). The trait was, however, highly heritable (0.93) and having a genetic advance of 20.03%, making it an important selection trait to enhance grain yield (Bhave *et al.,* 2016).

Table 2: Genetic parameters of morphological and phenological traits in proso millet.

Trait	Maximum	Minimum	Mean	PCV (%)	GCV (%)	H_2 (Broad sense)	GA (% mean)
Plant height	106.95	72.93	93.28	11.85	11.57	0.95	23.26
50% flowering	56.19	44.52	49.57	6.10	5.56	0.83	10.44
No. of leaves	9.89	6.56	8.39	10.98	10.48	0.91	20.60
Flag leaf length	25.49	19.44	22.83	8.74	8.53	0.95	17.16
Flag leaf width	1.32	1.01	1.17	8.01	7.68	0.92	15.20
Internodal length	8.60	8.70	7.47	12.30	12.09	0.96	24.48
No. of tillers	5.75	3.92	4.70	12.72	12.48	0.96	25.25
Panicle length	25.39	20.25	21.99	6.92	6.61	0.91	13.01
Days to maturity	97.52	80.72	87.60	5.65	5.14	0.82	9.63
1000 - Grain weight	7.40	5.18	6.35	10.38	10.05	0.93	20.03

CONCLUSION

The study revealed that several traits exhibited high genetic variability and heritability, implying good potential for improvement through selection. Traits such as internode length, number of tillers, plant height and flag leaf traits were particularly responsive to mutagenic treatments and showed high genetic advance, indicating the effectiveness of the induced variation.

Among treatments, Treatment 6 stood out with the tallest plants, highest internode length and highest 1000-grain weight. Treatment 7 excelled in panicle length and number of leaves, while Treatment 2 showed superior flag leaf length. These findings suggest that specific treatments have enhanced desirable traits and should be considered for further advancement in breeding programs.

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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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