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EMS-induced Development of Early-maturing Mutants in M_3 Generation of Barnyard Millet (*Echinochloa frumentacea* L.)

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ABSTRACT

Background: *Echinochloa frumentacea* (Barnyard millet) is a nutritionally important millet crop with limited genetic variability for crop improvement. The present study aimed to induce genetic variability through Ethyl methanesulfonate (EMS) mutagenesis and develop stable early-maturing mutants in the PRJ-1 variety.

Methods: Seeds of PRJ-1 were treated with EMS at 0.4%, 0.6%, 0.8% and 1.4% concentrations and evaluated across M_1, M_2 and M_3 generations under a randomized block design with three replications during 2022-2025 at Shri Guru Ram Rai University, Dehradun, Uttarakhand. Growth, germination and yield-related traits were recorded and analysed statistically.

Result: EMS treatments induced significant variability among the studied traits. The 0.6% EMS treatment recorded the highest germination (95.33%), root length (22.07 cm), shoot length (72.67 cm) and plant height (79.33 cm), showing significant improvement over the control ($p<0.05$). Higher EMS concentrations (0.8% and 1.4%) adversely affected germination and growth, indicating mutagenic toxicity. A stable early-maturing mutant line was identified in the M_3 generation, exhibiting flowering initiation at 40-45 days after sowing (DAS) and physiological maturity at approximately 65 DAS compared with 90-100 days in the control.

Key words: Barnyard millet, Early maturity, *Echinochloa frumentacea,* EMS mutagenesis, Mutation breeding.

INTRODUCTION

Barnyard millet (*Echinochloa frumentacea*) is an important small millet belonging to the Poaceae family and is widely cultivated in Asia, particularly in India, China and Japan, due to its adaptability to diverse agro-ecological conditions and its high nutritional value (Muthamilarasan and Prasad, 2015). The crop is increasingly recognized for its ability to grow under marginal conditions such as drought-prone and low-fertility soils, where major cereals often fail to perform effectively (Vetriventhan *et al*., 2023). It is a nutritionally rich cereal containing high levels of dietary fibre, protein, minerals and micronutrients, contributing significantly to food and nutritional security in developing countries (Saleh *et al*., 2013).

Despite these advantages, genetic improvement in barnyard millet has been limited compared with major cereals such as rice and wheat (Parry *et al.,* 2012). This limitation is largely due to the narrow genetic variability within cultivated germplasm, which restricts the efficiency of conventional breeding approaches (Mba, 2013). Therefore, alternative strategies such as mutation breeding have been explored to create novel genetic variability in crop plants (Muthamilarasan and Prasad, 2015; Khursheed *et al.,* 2023). Mutation breeding is an effective approach for inducing heritable genetic changes through physical or chemical mutagens (Parry *et al*., 2012). Among these, Ethyl methanesulfonate (EMS) is widely used because it induces point mutations at high frequency with relatively low chromosomal damage (Talebi *et al*., 2012). EMS causes alkylation of guanine bases in DNA, leading to GC-to-AT transitions that can alter gene function and result in phenotypic variation. EMS mutagenesis has been successfully used in cereals and millets to generate variability in traits such as plant height, yield components, stress tolerance and flowering time (Muthamilarasan and Prasad, 2015; Jerish *et al.,* 2025). In crops like rice, wheat, barley and sorghum, mutation breeding has contributed to the development of improved varieties with enhanced productivity and adaptability (Parry *et al.,* 2012). Early maturity is a key target trait in crop improvement, especially under rainfed and climate-variable conditions (Shekar *et al*., 2017). Early maturing genotypes complete their life cycle in a shorter duration, require less water and enable multiple cropping within limited time frames (Shadakshari *et al.,* 2001; Bentota, 2006; Domingo *et al.,* 2007; Rashid *et al.,* 2009). In this context, the barnyard millet genotype PRJ-1 was observed to exhibit promising

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performance by exhibiting early flowering at 45 DAS after sowing and attaining maturity within 65 DAS, compared to longer-duration controls.

In Barnyard millet, EMS mutagenesis has been reported to induce morphological and physiological variations including changes in plant height, leaf morphology, inflorescence traits and spikelet number (Ramesh *et al.,* 2019; Mensah and Obadoni, 2007). Moderate EMS doses often produce beneficial mutations, whereas higher doses may reduce germination and seedling vigour due to mutagenic damage (Talebi *et al.,* 2012). Seed germination and seedling growth parameters such as root length and shoot length are important indicators of the physiological effects of mutagen treatments. Mutagens influence cellular metabolism, enzyme activity and DNA replication, thereby affecting plant growth and development (Parry *et al.,* 2012). Moderate EMS concentrations may enhance growth, while higher concentrations can inhibit development due to chromosomal aberrations and metabolic disturbances (Talebi *et al*., 2012). Despite the successful use of EMS mutagenesis in several cereals and millets, information on the development of stable early-maturing mutants in barnyard millet remains limited. Moreover, no report is available on EMS-induced early-maturing mutants in the PRJ-1 variety. Therefore, the present study was undertaken to induce genetic variability using different EMS concentrations and identify stable early-maturing mutants in the M_3 generation, while evaluating their effects on growth, morphological and yield-related traits.

MATERIALS AND METHODS

Experimental location

The experiment was conducted during the *kharif* seasons of 2022-2025 at the research field, School of Agricultural Sciences, Shri Guru Ram Rai University, Pathribagh Campus, Dehradun, Uttarakhand, India (Fig 1). The experimental field consisted of sandy loam soil with a pH ranging from 6.8 to 7.2. Recommended agronomic practices were followed throughout the crop growth period. Fertilizers were applied following standard nutrient management recommendations using NPK fertilizer (10:5:10 ratio) to ensure optimum crop growth and development. Irrigation, weeding and plant protection measures were carried out uniformly across all treatments.

Plant material

The plant material used in the present study consisted of the barnyard millet (*Echinochloa frumentacea*) genotype PRJ-1. This genotype is a certified variety obtained from the Uttarakhand Seeds and Tarai Development Corporation Limited (UKS and TDC), which is known for maintaining improved crop varieties suitable for cultivation in northern India. The seeds used for the experiment were healthy, uniform and free from mechanical damage (Fig 2).

Mutagen treatment

Healthy and uniform seeds of the barnyard millet variety PRJ-1 were selected for the mutagenic treatment. Prior to EMS exposure, total of 100 healthy seeds was counted and were pre-soaked in distilled water for 12 hours to facilitate hydration and enhance the penetration of the mutagen into the seed and embryo activation also the tissues. The pre-soaked seeds were then subjected to EMS treatment using four concentrations (0.4%, 0.6%, 0.8% and 1.4%) prepared in phosphate buffer. The seeds were exposed to the EMS solution for 6 hours, which was standardized as the optimal treatment period to ensure maximum germination while maintaining effective mutagenic activity that was performed in dosage and chemical treatment time period selection before giving

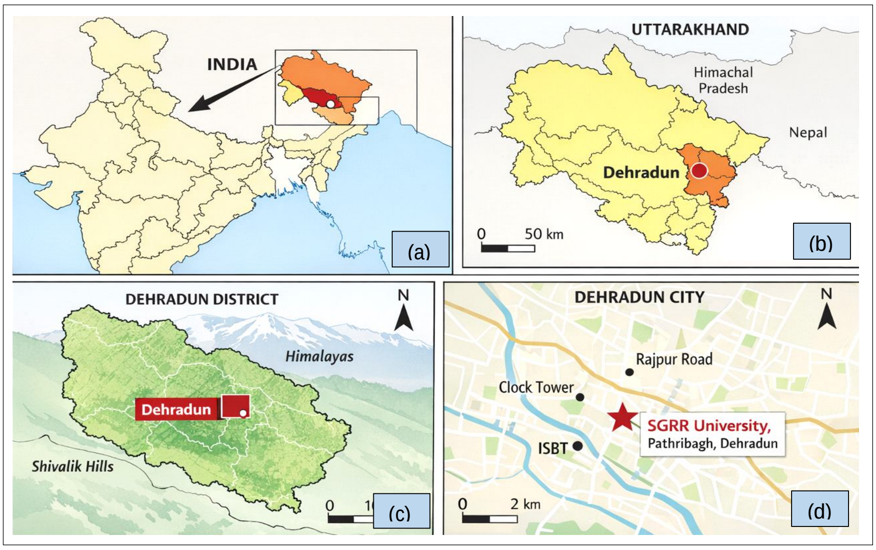

Fig 1: The location map showing the experimental site at Shri Guru Ram Rai University, Pathribagh, Dehradun, Uttarakhand, India.

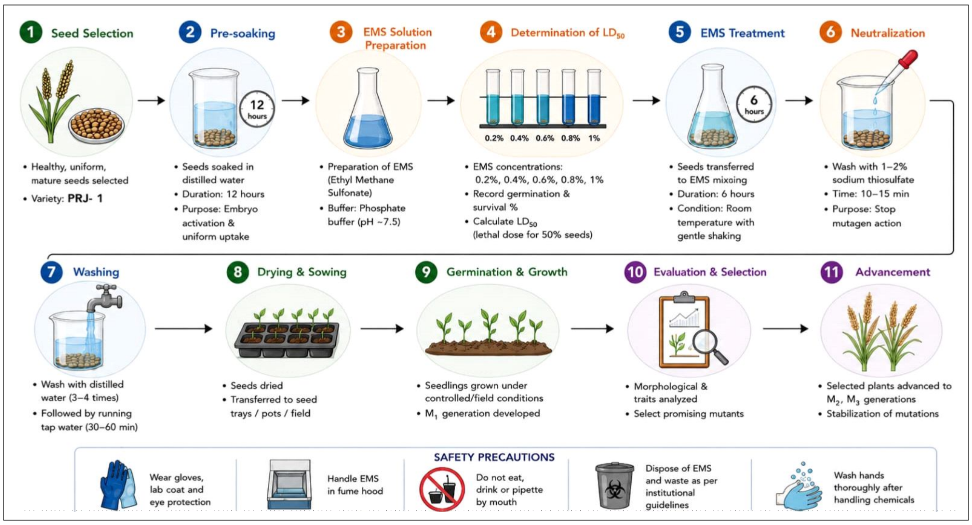

Fig 2: Procedure for inducing variation using EMS in barnyard millet.

Fig 3: The layout (2 m × 2 m) used for sowing seeds of PRJ-1 variety.

treatments (Fig 2). These concentrations were selected to represent a gradient of mutagenic intensity, allowing assessment of dose-dependent effects on germination, growth and morphological traits (Gosai *et al*., 2026; Laskar and Khan, 2017).

Following mutagenic treatment, the seeds were immediately subjected to a neutralization process using sodium thiosulphate to terminate residual EMS activity, which is essential to prevent excessive cellular damage (Roychowdhury *et al*., 2011). There after, seeds were thoroughly rinsed with distilled water and washed under running tap water to remove any remaining traces of EMS and sodium thiosulphate, ensuring minimal toxicity and safe handling (Kumari *et al*., 2024). After washing, the treated seeds along with control seeds were air-dried. Under controlled laboratory conditions, each treatment consisted of 100 seeds per replication. The seeds were then sown in prepared experimental plots following a randomized block design (RBD) with three replications for each concentration and control to ensure statistical reliability. The experimental material was subsequently advanced through successive generations (M_2 and M_3) using a dosage selection method to evaluate mutagenic effects and identify stable mutants.

The resulting plants were carefully monitored to record germination percentage, growth parameters, morphological traits and induced phenotypic variations across generations. This systematic approach facilitates the identification of useful mutants for crop improvement, genetic studies and sustainable agriculture (Food and Agriculture Organization, 2021).

Experimental design and field layout

Land was prepared with proper ploughing followed by application of farm yard manure to form 15 beds of 2 m × 2 m size. Suitable agricultural practices like irrigation, weeding was carried out for proper growth of the populations. The treated and control seeds were sown in raised bed in the month of April. Standard crop management practices such as irrigation, weed control and nutrient management were followed throughout the crop growth period (Fig 3).

Traits observed

Observations were recorded at 15, 45 and 65 DAS (Days after sowing) to evaluate the effect of different EMS concentrations on plant growth and development. The traits assessed included germination percentage, root length, shoot length, plant height, leaf breadth, leaf length, stem breadth, inflorescence length, spikelet number, seed yield per plant and flowering initiation. These parameters were selected to assess seedling vigour, vegetative growth, reproductive performance and the expression of early maturity in the treated populations.

Statistical analysis

The experimental data generated across M_1, M_2 and M_3 generations were subjected to statistical analysis using analysis of variance (ANOVA) under a randomized block design (RBD) with three replications. Mean values and standard error (SE) were computed for all quantitative parameters, including germination percentage, root length, shoot length, plant height, leaf traits and yield attributes. Treatment means were compared using the critical difference (CD) test at 5% level of significance to determine statistically significant differences among EMS concentrations and control.

To further understand the relationships among traits, correlation analyses were performed. Pearson's correlation coefficients were calculated to assess the degree of association between key growth and yield parameters such as root length, shoot length, plant height and seed yield.

The dose-dependent effects of different EMS concentrations (0.4%, 0.6%, 0.8% and 1.4%) were assessed by comparing treatment means with control values and consistency of trait expression across generations was used as an indicator of mutant stability. All statistical analyses were performed using Jamovi software and significance was tested at the 5% probability level. Treatment effects were considered significant at $p \leq 0.05$ and F-values, p-values and CV (%) were used to assess treatment differences and experimental precision (Gomez and Gomez, 1984; Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

Induced mutagenesis was carried out using the chemical mutagen-Ethyl methanesulfonate (EMS), which is one of the most commonly used alkylating agents in plant mutation breeding due to its efficiency in generating a high frequency of point mutations in DNA (Talebi *et al*., 2012; Hasan and Jagadish, 1992).

In the present study on the analysis of variance (ANOVA) revealed significant differences among treatments for most of the studied characters, indicating the effectiveness of EMS in inducing variability in *Echinochloa frumentacea* (Table 1). Highly significant treatment effects (P<0.01) were observed for root length, shoot length,

Table 1: ANOVA showing mean squares, F-values, probability levels, critical difference and coefficient of variation for different characters in *Echinochloa frumentecea.*

Trait	MS Treatment	MS error	F value	P value	CD 5%	CD 1%	CV (%)
RL_15	21.25**	0.228	93.145	0.001	0.8994	1.3085	4.39
RL_30	15.51**	0.205	75.61	0.001	0.8528	1.2408	3.26
RL_45	31.42**	1.329	23.63	0.0002	2.2713	3.159	5.94
SL_15	77.27**	9.325	8.285	0.006	5.7498	8.3654	17.97
SL_30	266.25**	7.381	36.071	0.001	5.1154	7.4424	8.14
SL_45	181.57**	8.816	20.594	0.003	5.5907	8.1339	4.82
G%_15	289.73**	7.933	36.521	0.001	5.3032	7.7157	3.14
G%_30	177.17**	10.116	17.512	0.0005	5.9887	8.713	3.68
G%_45	207.90**	17.4	11.948	0.0018	7.854	11.427	4.7
PH_15	54.30**	0.636	85.281	0.001	1.5023	2.1858	5.62
PH_30	279.98**	12.474	22.444	0.0002	6.6501	9.6752	10.27
PH_45	209.57**	13.116	15.977	0.0007	6.8191	9.9211	5.57
LB_15	0.017*	0.004	4.5217	0.033	0.1166	0.1696	18.91
LB_30	0.04	0.011	3.272	0.072	0.1975	0.2873	19.97
LB_45	0.23**	0.023	9.828	0.004	0.2876	0.4184	19.05
LL_15	1.44	0.504	2.862	0.096	1.3362	1.9441	15.17
LL_30	7.89	2.214	3.564	0.059	2.8018	4.0763	19.25
LL_45	15.10*	2.778	5.435	0.021	3.1386	4.5663	13.74
SB_15	0.02	0.017	1.176	0.389	0.2455	0.3572	39.4
SB_30	0.04	0.022	1.895	0.204	0.2814	0.4094	25.91
SB_45	0.13**	0.016	7.795	0.007	0.2406	0.3501	17.81
IL_15	0	0	-	-	0	0	-
IL_30	31.43**	2.35	13.36	0.001	2.8877	4.2013	28.57
IL_45	63.43*	9.73	6.52	0.012	5.8741	8.5463	29.29
SN_15	0	0	-	-	0	0	-
SN_30	44.50**	5.53	8.05	0.006	4.4262	6.4397	28.55
SN_45	79.27*	18.96	4.18	0.041	8.1999	11.93	28.86
SYPP_65	2.67	1.314	2.03	0.183	2.1582	3.14	43.96
FL_45	482.18*	121.90	3.95	0.047	20.788	30.245	29.95

Note: CT= Treatment, RL= Root length, SL= Shoot length, PH= Plant height, LB= Leaf breadth, LL= Leaf length, SB= Stem breadth, IL= Inflorescence length, SN = Spikelet number, SYPP= Seed yield per plant, FL= Date of initiation of flowering, SE= Standard error, **= Significant at 1%, *= Significant at 5%, NS= Non significant.

germination percentage, plant height, leaf breadth at 45 DAS, spikelet number at 30 DAS and inflorescence length at 30 DAS. Significant differences at the 5% level were recorded for leaf breadth at 15 DAS, leaf length at 45 DAS, inflorescence length at 45 DAS, spikelet number at 45 DAS and flowering initiation. Non-significant differences were observed for certain traits such as leaf length at 15 and 30 DAS, stem breadth at 15 and 30 DAS, seed yield per plant and early-stage reproductive traits. The coefficient of variation (CV) ranged from 3.14% for germination percentage at 15 DAS to 43.96% for seed yield per plant, indicating varying levels of experimental variability among the traits studied.

Present study revealed significant variations in growth and morphological parameters of barnyard millet under different EMS treatment concentrations at 15, 45 and 65 DAS (Table 2). Moderate EMS treatments showed a stimulatory effect on plant growth, whereas higher concentrations exhibited inhibitory effects. Among all treatments, 0.6% EMS concentration consistently recorded the highest values for most growth parameters. Root length (RL) increased progressively with plant age, reaching maximum values at 65 DAS, with the highest observations in the 0.6% treatment across all replications. Similarly, shoot length (SL) and plant height (PH) were significantly enhanced under 0.6% EMS, indicating improved vegetative growth compared to control and other treatments. The germination percentage (G%) was also highest in the 0.6% treatment (ranging up to 98%), suggesting that this concentration was optimal for seed viability and early establishment. In contrast, higher EMS concentration (1.4%) showed reduced germination and growth performance, indicating toxic effects at elevated doses (Table 2).

Leaf parameters, including leaf breadth (LB) and leaf length (LL), showed gradual increases with plant age and were comparatively higher under 0.4% and 0.6% treatments. The stem breadth (SB) followed a similar trend, with moderate treatments promoting thicker stems, which is indicative of better plant vigour.

Reproductive parameters such as inflorescence length (IL) and spikelet number (SN) were also positively influenced by moderate EMS doses. The 0.6% treatment produced longer inflorescences and a higher number of spikelet, reflecting improved reproductive efficiency. In contrast, higher concentrations (0.8% and 1.4%) resulted in reduced reproductive traits. Seed yield (SY) was found to be highest in the 0.6% treatment across replications, further confirming its effectiveness in enhancing overall plant productivity. Lower and higher concentrations showed comparatively reduced yields. With respect to phenology, flowering (FL) was observed around 40-45 DAS, with slight variation among treatments. Early flowering was more prominent in the 0.6% treatment (around 40 DAS), whereas delayed flowering (up to 45 DAS) was observed in higher EMS concentrations. The crop attained physiological maturity at approximately

Table 2: Effect of different treatment concentrations on growth and morphological parameters at 15, 45 and 65 DAS.

CT	R	RL			SL			G%			PH			LB			LL			SB			IL			SN			SYPP	FL
DAS		15	45	65	15	45	65	15	45	65	15	45	65	15	45	65	15	45	65	15	45	65	15	45	65	15	45	65	65	40-45
C	Mean	5.60	8.83	13.63	9.3	15.87	54.33	79.33	90.67	93.33	10.43	18.77	59	0.27	0.4	0.43	3.33	6.8	10.27	0.33	0.5	0.7	0	1.93	4	0	2.67	5	1.13	14.33
	SE	0.53	0.35	0.67	0.56	0.31	4.04	1.15	1.15	2.89	0.40	0.93	6.56	0.12	0.1	0.12	0.42	0.3	0.31	0.25	0.26	0.26	0	3.35	6.93	0	4.62	8.66	1.95	24.83
0.4%	Mean	8.57	12.77	18.3	11.27	32.37	62	92.67	93	95.67	13.1	37.27	66	0.27	0.4	0.97	3.8	8.23	12.67	0.27	0.53	0.77	0	6.93	10.67	0	8.33	13.67	2.92	42.67
	SE	0.45	0.17	0.41	0.96	3.19	1.63	1.89	1.41	1.25	0.42	4.97	3.27	0.05	0.08	0.17	0.28	0.61	0.94	0.09	0.05	0.05	0	0.61	0.94	0	0.94	1.25	0.38	0.47
0.6%	Mean	12.9	15.2	22.07	17.03	42.1	72.67	90	91.67	95.33	19.6	45.3	79.33	0.33	0.57	1.1	4.9	10.63	16.33	0.33	0.57	0.93	0	9.33	14.33	0	12.37	19	3.73	40.33
	SE	0.3	0.2	1.69	1.93	1.54	2.52	0	1.53	3.06	0.92	0.95	1.15	0.06	0.15	0.1	0.75	1.66	2.52	0.06	0.15	0.06	0	0.40	0.58	0	0.65	1	0.62	0.58
0.8%	Mean	10.3	12.03	16.37	19.9	32.13	63.67	79.67	83.33	84.33	14.3	35.77	68	0.2	0.47	0.7	4.7	10.17	13.67	0.27	0.43	0.7	0	6.1	9.33	0	8	13	2.58	42.67
	SE	0.7	0.85	0.67	6.61	3.61	3.21	0.58	4.16	5.86	0.4	3.60	0	0.00	0.06	0.1	0.96	2.08	1.53	0.06	0.06	0.1	0	0.35	0.58	0	1	1	0.37	1.15
1.4%	Mean	8.9	12.23	15.3	20.5	31.13	53.67	68	74.67	76.67	20.27	33.97	59	0.13	0.27	0.57	3.6	7.8	12	0.13	0.27	0.37	0	10.17	15.67	0	4	15.33	2.67	44.33
	SE	0.3	0.35	1.2	0.46	3.48	1.53	5.29	4.16	5.77	1.08	3.26	1	0.06	0.06	0.29	0.6	1.3	2	0.06	0.06	0.06	0	0.97	1.53	0	1	2.52	1.15	1.15

Note: CT= Treatment, DAS= DAS after sowing, C= Control, RL= Root length, SL= Shoot length, PH= Plant height, LB= Leaf breadth, LL= Leaf length, SB= Stem breadth, IL= Inflorescence length, SN= Spikelet number, SYPP= Seed yield per plant, FL= Date of initiation of flowering, SE= Standard error.

65 DAS, confirming the early maturing nature of the treated variants. The 0.6% EMS treatment recorded significantly higher values ($p \leq 0.05$) for germination, root length, shoot length, plant height and yield-related traits compared with the control and other EMS treatments.

Overall results clearly demonstrate that EMS induces dose-dependent effects on plant growth and development. A significant increase ($p<0.05$) in growth parameters was observed under 0.6% EMS treatment emerged as the most effective, significantly enhancing germination, vegetative growth and yield attributes as per prior studies reported by Kavera *et al.* (2017). This increase was 35-40% higher than control. In contrast, higher concentrations (1.4%) exhibited inhibitory effects, reducing growth and productivity. In contrast, the reduction in growth and germination observed at higher EMS concentrations (1.4%) may be associated with mutagenic toxicity. Similar inhibitory effects of higher EMS doses have been reported in several cereals and legumes (Talebi *et al*., 2012; Wani *et al*., 2012). This dual role of EMS as both a mutagenic and cytotoxic agent has been well documented in the literature, where optimal doses induce useful genetic variability, while excessive doses cause detrimental effects (Gaul, 1964; Wani *et al*., 2012). Therefore, the identification of an optimal EMS concentration is crucial for successful mutation breeding and crop improvement programs. The stimulatory effect observed at moderate EMS concentrations, particularly 0.6%, may be attributed to the induction of beneficial genetic variability without causing excessive cellular damage. Moderate EMS doses can enhance metabolic activity, cell division and physiological efficiency, resulting in improved germination, seedling vigour and vegetative growth. Similar improvements following moderate EMS treatments have been reported in rice, groundnut, soybean and other millet crops (Talebi *et al*., 2012; Kavera and Nadaf, 2017; Shekar and Pushpendra, 2017).

Seed vigour Index, is widely used to evaluate seedling performance by integrating germination percentage with seedling growth parameters. It provides an overall assessment of seed quality and early plant development. In the present study, seed vigour index was calculated using the following relationship:

$$SVI = (\text{Root length} + \text{Shoot length}) \times \text{Germination}\%$$

SVI integrates both germination and seedling growth, providing a comprehensive measure of seed vigour

Results indicated that moderate EMS concentrations produced higher seed vigour index values compared with both control and higher EMS treatments. The highest vigour index was observed under 0.6% EMS treatment, indicating superior seedling vigour and growth performance. These findings suggest that optimal EMS concentrations can enhance early seedling development by inducing beneficial genetic variations. Observed enhancement at moderate EMS doses aligns with previous mutation breeding studies where moderate EMS doses improved seedling vigour and growth potential, while higher doses caused physiological damage leading to reduced vigour (Talebi *et al.,* 2012; Ramesh *et al.,* 2019).

The present analysis demonstrates the effect of different concentrations of Ethyl methanesulfonate (EMS) on the inflorescence traits of the PRJ-1 variety of barnyard millet (Fig 4).

The control (0%) exhibited normal morphology, while varying EMS doses induced noticeable changes in spikelet number and inflorescence structure. Among the treatments, the 0.6% EMS concentration showed the most pronounced positive effect, producing a comparatively bulkier and more compact inflorescence with increased spikelet number, indicating enhanced reproductive potential. Lower concentrations (0.4%) resulted in moderate variations, whereas the higher concentration (0.8%, 1.4%) appeared to exert inhibitory effects, likely due to mutagenic toxicity affecting normal growth processes. Overall, the results suggest a dose-dependent response of EMS, with 0.6% identified as the optimal concentration for inducing beneficial morphological variations in barnyard millet (Fig 4).

Statistical analysis (**Scattered plots)**

The statistical analysis as per (Table 2) to examine the relationship between two continuous variables by representing individual data points on a Cartesian plane. They provide a clear visual understanding of the direction, strength and pattern of association between variables, which is essential prior to conducting correlation or regression analysis. In biological and agricultural studies, scatter plots are particularly useful for assessing relationships among morphological traits such as root length, shoot length and plant height. For instance, a positive linear trend in a scatter plot indicates that an increase in one trait is associated with an increase in another, reflecting coordinated growth patterns. Additionally,

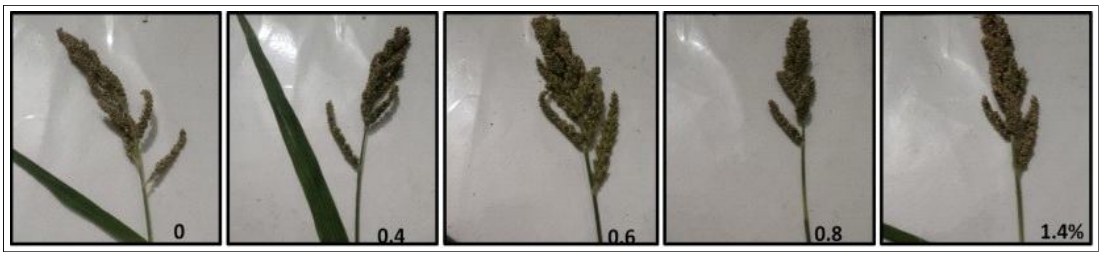

Fig 4: Inflorescence of 0% (control), 0.4%, 0.6%, 0.8% and 1.4% EMS treatment showing variations.

scatter plots help in identifying outliers and data clusters, which may indicate variability among treatments or experimental conditions. According to Montgomery *et al*. (2021) scatter plots are fundamental tools for preliminary data exploration and model assessment, while Field (2013) emphasized their importance in detecting relationships and validating assumptions of statistical tests. Thus, scatter plots serve as an essential graphical method for understanding interrelationships among variables and supporting further statistical analysis in experimental research (Field, 2013; Montgomery *et al.,* 2021).

Root length vs shoot length the scatter plot depicting root length (RL) versus shoot length (SL) shows a clear positive relationship, indicating that an increase in root growth is associated with a corresponding increase in shoot growth (Fig 5). The data points for the 0.6 treatment are clustered at higher RL and SL values, demonstrating superior performance and strong coordination between below- and above-ground growths. In contrast, the 1.4 treatment is positioned at comparatively lower values, indicating reduced growth and possible inhibitory effects at higher concentration. The control exhibits the lowest cluster, reflecting minimal growth. Overall, this graph suggests that moderate treatment (0.6) enhances root-shoot interaction, leading to improved plant development.

The scatter plot of PH versus LL reveals a positive association between these two traits, suggesting that taller plants tend to develop longer leaves (Fig 6). The 0.6 treatment shows the highest clustering, indicating maximum plant height and leaf expansion, which reflects enhanced vegetative growth. Conversely, the 1.4% treatment exhibits relatively lower values, suggesting restricted growth possibly due to stress or toxicity at higher concentration levels. The control group remains at the lower end of the distribution. This relationship highlights that optimal treatment levels improve both structural growth and leaf development, contributing to better photosynthetic capacity.

The scatter plot illustrating RL versus PH demonstrates a positive correlation indicating that plants with longer roots tend to achieve greater height (Fig 7). The 0.6% treatment is clearly associated with higher RL and PH values, confirming its effectiveness in promoting overall plant vigour. In contrast, the 1.4% treatment is positioned lower on both axes, indicating weaker root development and reduced plant height. The control group shows the least growth. This graph emphasizes the importance of root development in supporting vertical growth and confirms that moderate treatment enhances overall plant performance, while higher concentrations may hinder growth.

The scatter plot of SL versus PH indicates a strong positive linear relationship, demonstrating that increased shoot elongation contributes directly to greater plant height (Fig 8). The 0.6% treatment exhibits the highest values for both SL and PH, reflecting optimal growth conditions and efficient biomass accumulation. In contrast, the 1.4% treatment shows comparatively lower values, indicating

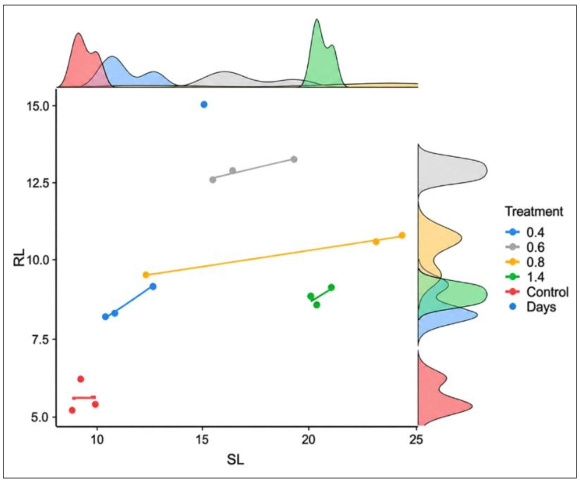

Fig 5: Relationship between shoot length (SL) and root length (RL) under different treatment concentrations.

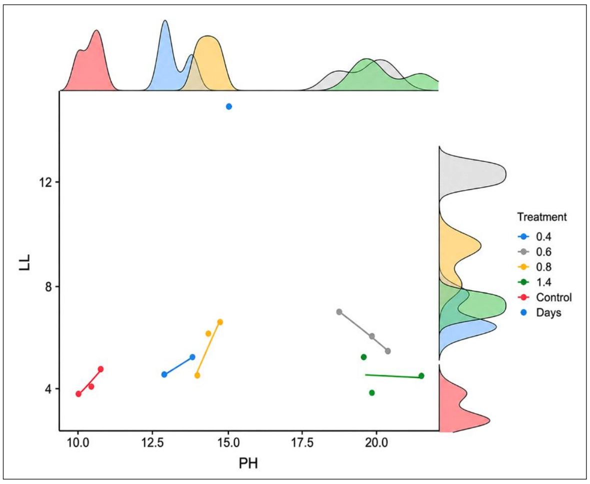

Fig 6: Relationship between leaf length (LL) and plant height (PH) under different treatment concentrations.

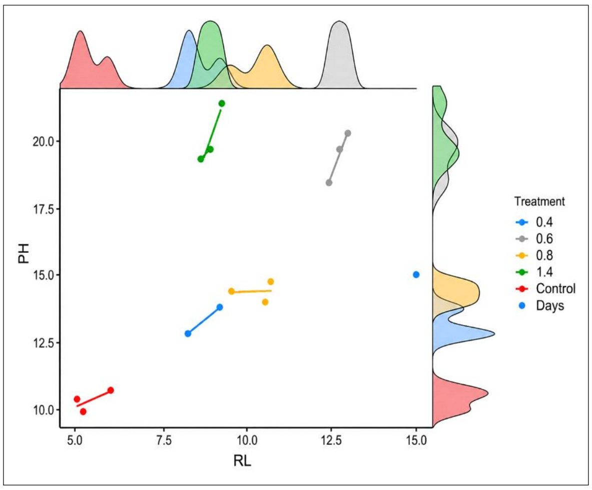

Fig 7: Relationship between plant height (PH) and root length (RL) under different treatment concentrations.

restricted shoot development and reduced plant height. The control group again remains at the lowest range. This graph confirms that shoot growth is a major determinant of plant height and that moderate treatment levels significantly enhance above-ground growth, while higher doses have an inhibitory effect.

The Pearson correlation heatmap indicated positive associations among several growth, morphological and yield-related traits. Correlation coefficients ranged from moderate to strong positive values, suggesting possible relationships among these characters. However, these associations should be interpreted cautiously because correlation does not imply causation and further validation is required under larger populations and multiple environments. As per (Fig 9), the intensity of green colour indicates strong positive correlation, while lighter shades or near-zero values indicate weak or negligible relationships. A strong positive correlation was observed among most morphological and yield traits. Parameters such as LB, LL, SB, IL and SN improvement in one trait is likely to be associated with improvement in others. This suggests that improvement in one of these traits is likely to be associated with improvement in others, indicating their collective contribution toward overall plant productivity. SY exhibited positive associations with SN and IL suggesting that reproductive traits may contribute to yield improvement. However, the magnitude of these correlations should be interpreted carefully and additional studies involving larger populations are required to validate these relationships. RL and SL exhibited moderate positive correlations with most traits ($r \approx 0.50$-0.68), suggesting their importance during early growth stages in establishing overall plant Vigour. However, their association with reproductive traits was comparatively lower than that of morphological parameters. PH showed weak or negligible correlations with several parameters ($r \approx -0.04$ to 0.05), indicating that plant height alone may not be a reliable indicator of yield or reproductive success in this study. Correlation analysis reveals that reproductive and morphological traits are strongly interlinked and selection based on these traits particularly inflorescence length, spikelet number and leaf characteristics can be effective for improving seed yield in barnyard millet. The correlation analysis indicates that EMS-induced variability influenced several growth, morphological and yield-related traits. Nevertheless, correlation does not establish causation and the observed associations should be considered indicative rather than conclusive. The development of early-maturing mutants has important practical implications for crop improvement. Early-maturing genotypes can escape terminal drought, require fewer production inputs, facilitate multiple cropping systems and improve adaptation under changing climatic conditions. Therefore, the identified early-maturing mutant lines may serve as valuable genetic resources for future barnyard millet breeding programmes aimed at enhancing productivity and climate resilience.

Identification and stability of early-maturing mutants

Early-maturing mutant lines were identified based on flowering initiation at 40-45 DAS and physiological maturity at approximately 65 DAS compared with the control plants, which required 90-100 days to attain maturity. The selected mutant lines exhibited consistent expression of early maturity and associated morphological traits across M_1, M_2 and M_3 generations, indicating phenotypic stability. Mutants were selected and advanced

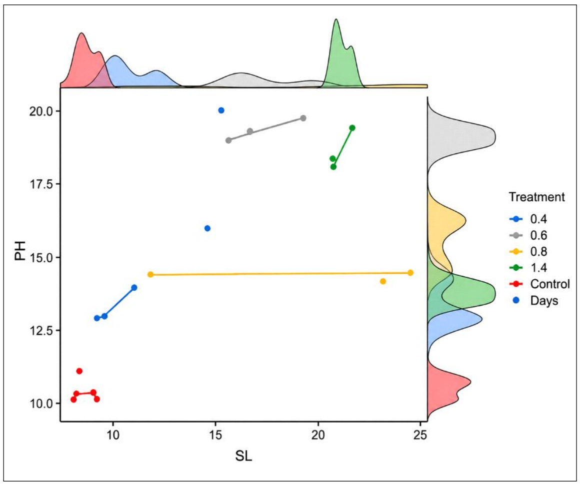

Fig 8: Relationship between plant height (PH) and shoot length (SL) under different treatment concentrations.

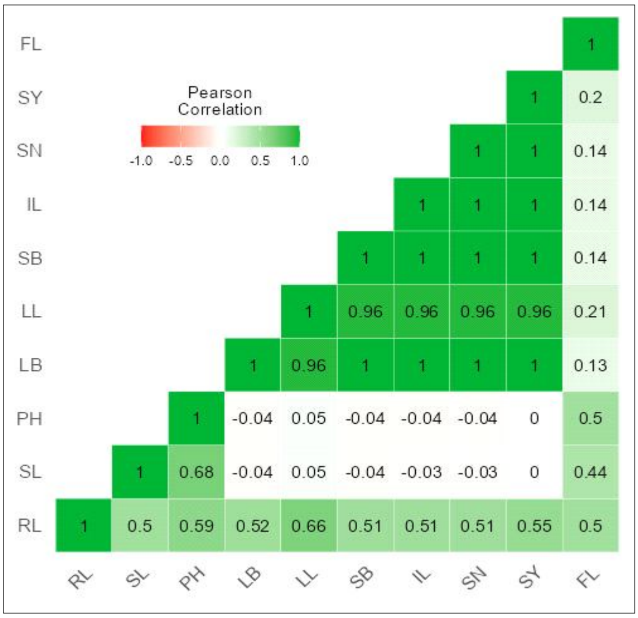

Fig 9: The pearsons correlation heatmap illustrates the correlation among various plant growth parameters.

individually in each generation based on earliness, plant vigour and desirable agronomic characteristics.

CONCLUSION

Study demonstrates that EMS induces significant dose-dependent variations in barnyard millet. Among all treatments, 0.6% EMS proved to be the most effective, enhancing germination, growth and yield-related traits, while higher concentrations showed inhibitory effects. An early maturing mutant was successfully developed, exhibiting flowering at 40-45 DAS and maturity at 65 DAS, compared to the control. The strong association among morphological and yield traits indicates effective induction of variability. Overall, EMS mutagenesis is an efficient tool for crop improvement and 0.6% is identified as the optimum dose for developing early maturing, high-performing barnyard millet genotypes.

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Disclaimers

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

Conflict of interest

Authors have declared that no competing interests exist.

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